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Protective Monotherapy Against Lethal Ebola Virus Infection by a Potently Neutralizing Antibody

Davide Corti,[1,4*] John Misasi,[2*] Sabue Mulangu,[2] Daphne A. Stanley,[2] Masaru Kanekiyo,[2] Suzanne Wollen,[3] Aurélie Ploquin,[2] Nicole A. Doria-Rose,[2] Ryan P. Staupe,[2] Michael Bailey,[2] Wei Shi,[2] Misook Choe,[2] Hadar Marcus,[2] Emily A. Thompson,[2] Alberto Cagigi,[2] Chiara Silacci,[1] Blanca Fernandez-Rodriguez,[1] Laurent Perez,[1] Federica Sallusto,[1] Fabrizia Vanzetta,[4] Gloria Agatic,[4] Elisabetta Cameroni,[4] Neville Kisalu,[2] Ingelise Gordon,[2] Julie E. Ledgerwood,[2] John R. Mascola,[2] Barney S. Graham,[2] Jean Jacques Muyembe-Tamfun,[5] John C. Trefry,[3†] Antonio Lanzavecchia,[1†] and Nancy J. Sullivan[2*†]

[1]Immune Regulation Unit
Institute for Research in Biomedicine
CH-6500 Bellinzona, Switzerland

[2]Vaccine Research Center
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, MD 20892 USA

[3]United States Army Medical Research Institute of Infectious Diseases
Fort Detrick, MD 21702 USA

[4]Humabs BioMed SA
6500 Bellinzona, Switzerland

[5]National Institute for Biomedical Research
National Laboratory of Public Health
Kinshasa B.P. 1197, Democratic Republic of the Congo

*These authors contributed equally to this work.
†These authors contributed equally to this work.

Corresponding Author: Nancy J. Sullivan
Vaccine Research Center, National Institutes of Health
40 Convent Drive, Bldg. 40/2509
Bethesda, Maryland 20892, USA
Phone number: 301-435-7853
njsull@mail.nih.gov

Abstract:

Ebola virus disease in humans is highly lethal, with case fatality rates ranging from 25-90%. There is no licensed treatment or vaccine against the virus, underscoring needs for efficacious countermeasures. Here, we demonstrate that human survivors of Ebola virus disease maintain circulating antibodies against the Ebola virus surface glycoprotein for more than a decade after infection, and we isolated monoclonal antibodies (mAb) from one survivor of the 1995 Kikwit outbreak in the Democratic Republic of Congo. These mAbs bind and neutralize recent and previous outbreak strains of Ebola virus, and mediate antibody-dependent cell-mediated cytotoxicity *in vitro*. Administration of a single potently neutralizing antibody, mAb114, protected infected macaques from death and clinical illness when administered one day after lethal challenge. Treatment with a single human mAb suggests a simplified therapeutic strategy for human Ebola infection may be possible.

One Sentence Summary:

A single neutralizing monoclonal antibody isolated from a human Ebola survivor completely protected non-human primates from lethal Ebola infection.

Main Text:

Ebola virus disease (EVD) causes severe illness characterized by rapid onset of fever, vomiting, diarrhea and bleeding diathesis (*1*, *2*), and was first described in the Democratic Republic of Congo in 1976 (*2*). The 2014 outbreak in West Africa has

affected over 27,000 and claimed at least 11,000 lives (*1*). The challenges of a large outbreak and the failure of traditional quarantine and contact tracing measures (*3*, *4*) to control this outbreak highlights the urgency for therapies. The success in nonhuman primates (NHP) of ZMapp, a cocktail of three mouse-human chimeric mAbs derived from immunized mice (*5*, *6*), illustrated the potential impact of monoclonal antibody therapies against EVD, and it is currently being evaluated in human trials. To date, efforts to simplify the ZMapp regimen to contain fewer mAbs have not been successful in the macaque EVD model (*7*). We sought to isolate mAbs from human survivors of Ebola virus infection, with the goal of identifying antibodies that confer clinical protection either as single or dual-combination agents.

We obtained blood from two survivors of the 1995 Kikwit EVD outbreak (*8*) eleven years after infection. These subjects were the sole survivors of a family of 15 people who were infected during the outbreak. At the time of infection, subject 1 (S1) was a male 28-year-old who had severe laboratory-confirmed illness and, following recovery, worked for several months in the EVD ward caring for other patients. His sister (S2) was 20-years old and had moderate disease severity that was clinically diagnosed based on contact history and symptoms. To determine if the subjects retained circulating antibodies against Ebola virus (EBOV) glycoprotein (GP), we assessed GP-specific antibodies by ELISA (Fig. 1A). We observed reciprocal EC_{90} titers of 2,326 and 275 in the sera of S1 and S2, respectively. Moreover, the serum from S1, the more severely ill subject, displayed potent virus neutralizing activity (Fig. 1B). The results indicated that these survivors maintained serologic memory against EBOV GP more than a decade

following infection, and suggested the potential to clone immunoglobulins with potent neutralizing activity from their memory B cells.

We focused on S1 due to high serum neutralizing activity, sorted IgG memory B-cells from his peripheral blood mononuclear cells (PBMC), and immortalized individual clones with Epstein-Barr virus (*9*). Forty immortalized clones whose supernatants displayed a range of GP-binding activity by ELISA were identified (Fig. 1C). Two clones, 100 and 114, expressed antibodies with markedly higher neutralizing activity than all others (Fig. 1D). A second immortalization yielded 21 clones, from which two additional GP-specific clones, 165 and 166, were rescued (Fig. S1).

Immunoglobulin sequences were PCR-amplified from the four clones and used to produce mAb100, mAb114, mAb165 and mAb166 by transient transfection. We assessed ELISA binding to EBOV GP and observed that one antibody, mAb114, stood apart from the others, displaying nearly 100% higher maximal binding (Fig. 2A). The remaining three antibodies, mAb100, mAb165 and mAb166, exhibited reduced levels of maximal binding compared to mAb114, but were comparable to each other and to KZ52, a prototype human EBOV GP-specific mAb (*10*). mAb114 achieved half maximal binding (EC_{50}) at a concentration of 0.07 μg/mL, which was up to two orders of magnitude lower EC_{50} than the other mAbs. mAb100 and mAb166 had similar binding profile (0.26 μg/mL, 0.40 μg/mL) while mAb165 bound less well with an $EC_{50} > 1$ μg/mL.

To test potential functional properties of the mAbs we evaluated inhibition of GP-mediated entry into HEK293T cells in the absence of complement (Fig. 2B, Fig. S2). mAb165 and mAb166 both neutralized well and exhibited similar potencies for half-maximal inhibition (IC_{50}) concentration of 1.77 and 0.86 μg/ml, respectively. mAb100 and mAb114 resided in the strongest neutralizing group, with IC_{50} about one-log greater (0.06 and 0.09 μg/ml, respectively) than mAb165 and mAb166. Notably, all four of the neutralizing antibodies inhibited 100% of the input virus unlike KZ52, which consistently displayed only 80-90% maximum inhibition, and 13C6 which neutralized < 20% at 10 μg/mL. Importantly, neutralization of the 2014 West African Makona variant was achieved within similar concentration ranges seen for the Mayinga variant (Fig. S3).

Sequence analysis revealed mAb114 and mAb165 to be IgG1 isotypes, and mAb100 and mAb166 to be IgG3 isotypes. Immunoglobulins displayed between 85-95% and 89-97% germline identity for heavy and light chains, respectively (Fig. 2C). Analyses of germline gene usage and V(D)J recombination indicate that they originate from different B-cell lineages. Interestingly, mAb114 utilizes *IGHV3-13*01*, a rarely used VH gene, and *IGKV1-27*01*.

We next analyzed the role of somatic hypermutations for the two most potent antibodies, mAb100 and mAb114, using variants that were partially or completely reverted to the unmutated common ancestors (UCAs) (Fig. 2, D to G). The fully reverted version of mAb100 (UCA/UCA), as well as a variant with germline VH and a VL with a single change from germline (A89T), recognized cells expressing GP with only a 2- to 4-fold

weaker binding compared with the fully matured antibody (Fig. 2H). GP binding comparable to the fully matured mAb100 heavy and light chains (sH/sL) was observed when three HCDR3 mutations (A96V/V103Y/Y114S) were introduced in the reverted germline antibody (gH/UCA), illustrating that those mutations were sufficient to mediate binding observed with fully matured mAb100. The addition of all the other mutations did not contribute further to mAb100 binding to GP. In the case of the mAb114, the fully reverted version of mAb114 (UCA/UCA) demonstrated negligible binding to EBOV GP (Fig. 2I). Introduction of two mutations (A96V and Y108S) in the HCDR3 of mAb114 germline was sufficient to confer an increase in GP binding. It is intriguing that these mutations (A96V and Y108S) are located at the base of the HCDR3 loop which are most likely not in direct contact with GP but may have a stabilizing effect on the whole HCDR3. Indeed, restoration to the binding equivalent of the mature antibody required a fully matured light chain in addition to the two HCDR3 mutations. Inherent uncertainty in determining the germline configuration of the HCDR3 does not appear to apply to this case since the two mutations are located in the V and J regions of the junction and no polymorphisms have been described at those positions. Importantly, the fully mutated light chain gene, as shown in the case of the mAb114 UCA/sK variant, can partially compensate for the lack of somatic mutation in the heavy chain (Fig. 2I). The presence of additional mutations on either VH or VK is required to achieve the level of the fully matured mAb114 binding. These results suggest a rapid pathway of mAb114 affinity maturation through one or two somatic mutations, which became redundant as further mutations accumulated, a finding that is reminiscent of what was recently observed for the generation of broadly neutralizing influenza antibodies (*11*).

Since mAb100 and mAb114 were the most potently neutralizing antibodies, they were considered optimal candidates for further evaluation. In order to assess the potential for synergy between these antibodies in the context of combination therapy, we wished to first rule out cross-competition for antigen binding or targeting of a single immunodominant region of GP. We found that each antibody bound to GP in the presence of the other, suggesting that they recognize distinct regions on GP (Fig. 3A) and therefore could be used together in combination immunotherapy to improve efficacy and diminish the likelihood of emergence viral escape mutants (*12*, *13*). To define the regions targeted by mAb100 and mAb114 we employed biolayer interferometry to assess GP binding in competition with mAbs KZ52 and 13C6, which have known epitopes in the GP base and glycan cap, respectively (*14*, *15*). We found that mAb100 competes with KZ52 for binding at the base of GP, while mAb114 recognizes at least in part the glycan cap region, as demonstrated by the partial competition observed with 13C6 (Fig. 3, B and C).

Since some EBOV GP antibodies have been suggested to mediate antibody dependent cell-mediated cytotoxicity (ADCC) (*16*) the ADCC activity of mAb100 and mAb114 were determined in a flow cytometry-based assay using GP-expressing target cells (Fig. 3D). We found that both mAb100 and mAb114 mediated ADCC, and maximum activity was observed at a mAb concentration of 0.03 μg/ml, which is similar to the IC_{50} values for neutralization. Killing of target cells was demonstrated to be mediated through Fc receptors since LALA mutations in the mAb Fc regions (*17*) of the antibodies abrogated

ADCC activity. Therefore, in addition to neutralization, these mAbs have the potential to induce direct killing of infected cells *in vivo*, a key viral clearance mechanism.

The presence of potent neutralizing and ADCC activity, and the absence of cross competition, supported testing mAb100 and mAb114 *in vivo* for protective efficacy in macaques. We challenged four rhesus macaques (*Macaca mulatta*) with a lethal dose of Ebola virus, Kikwit 1995 variant. One day post-challenge, the treatment group (n=3) was given an intravenous injection with a mixture of mAb100 and mAb114 at a total combined dose of 50 mg/kg, and the treatment was repeated twice more at 24-hour intervals (Fig. 4A). Circulating Ebola GP-specific antibody titers in the mAb recipients peaked after the second mAb injection and reciprocal ELISA titers remained above 10^5 throughout the study, suggesting minimal clearance of the mAbs during the observation period (Fig. 4B). The naive untreated macaque succumbed to EVD on day 10 with a circulating viral load exceeding 10^8 ge/ml (Fig. 4, C and D). In contrast, all three mAb-treated macaques survived challenge without detectable systemic viremia. Consistent with historic controls, the untreated animal displayed hallmark indicators of Ebola infection including hematologic, liver and renal dysfunction as indicated by thrombocytopenia and striking elevations in alanine transaminase (ALT) and creatinine from day 6 through the time of death (Fig. 4E, Figs. S4 to S7). In contrast, macaques in the treatment group remained within normal ranges for these parameters, and remained free of all EVD symptoms.

We next asked whether monotherapy is sufficient for protection of NHP, and focused on mAb114 since it showed higher maximal binding than mAb100. As in the first experiment we exposed four macaques to a lethal dose of EBOV and administered 50 mg/kg of mAb114 (n=3) to the treatment group after a one-day delay, followed by two more doses at 24-hour intervals. All treated macaques survived, whereas the control animal succumbed to EVD on day 6 with a peak viral load of 10^{10} ge/ml (Fig. 4, F to H). In contrast to the previous experiment, transient viremia was observed in the treated animals, but it remained at levels less than 0.1% of the untreated control animal, and returned to undetectable levels. Despite transient viremia, treated animals remained free of clinical and laboratory abnormalities (Fig. 4I, Figs. S8 to S11).

mAb114 has several characteristics that may contribute to protection as a monotherapy compared to KZ52 and 13C6, which were non-protective in NHPs (*7*, *18*). Firstly, both KZ52 and mAb114 neutralize with potent IC_{50s}, however mAb114 neutralizes 100% of input virus whereas KZ52 plateaus at 80-90%. Secondly, mAb114 does not require complement for neutralizing activity in contrast to 13C6 (Figure 2B) (*6*). Based on these observations, one intriguing hypothesis is that protective monotherapy requires both potent binding and complete neutralization in the absence of complement. In addition, ADCC activity may contribute to the unique ability of mAb114 to protect as a monotherapy against lethal Ebola infection of macaques.

In these studies, we showed that circulating functional antibodies as well as memory B cells specific to Ebola virus are maintained in survivors for more than a decade following

infection. mAbs isolated from a survivor of the 1995 Kikwit EVD outbreak exhibited ADCC activity and showed potent neutralizing activity against two other Ebola variants, including one from the recent West Africa outbreak. Macaques who received mAb114 and mAb100 as combination therapy remained healthy with no signs of viremia after EBOV challenge. Strikingly, when a single antibody, mAb114, was therapeutically administered after lethal EBOV challenge of macaques, all treated animals were fully protected and asymptomatic, despite a low transient level of circulating virus being detected. These results contribute to understanding the mechanisms of antibody-mediated Ebola virus protection, and suggest a simplified therapeutic option for EVD may be possible.

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Supplementary Materials

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Materials and Methods

Figs. S1-S11

References 18-23

Figures

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Figure 1

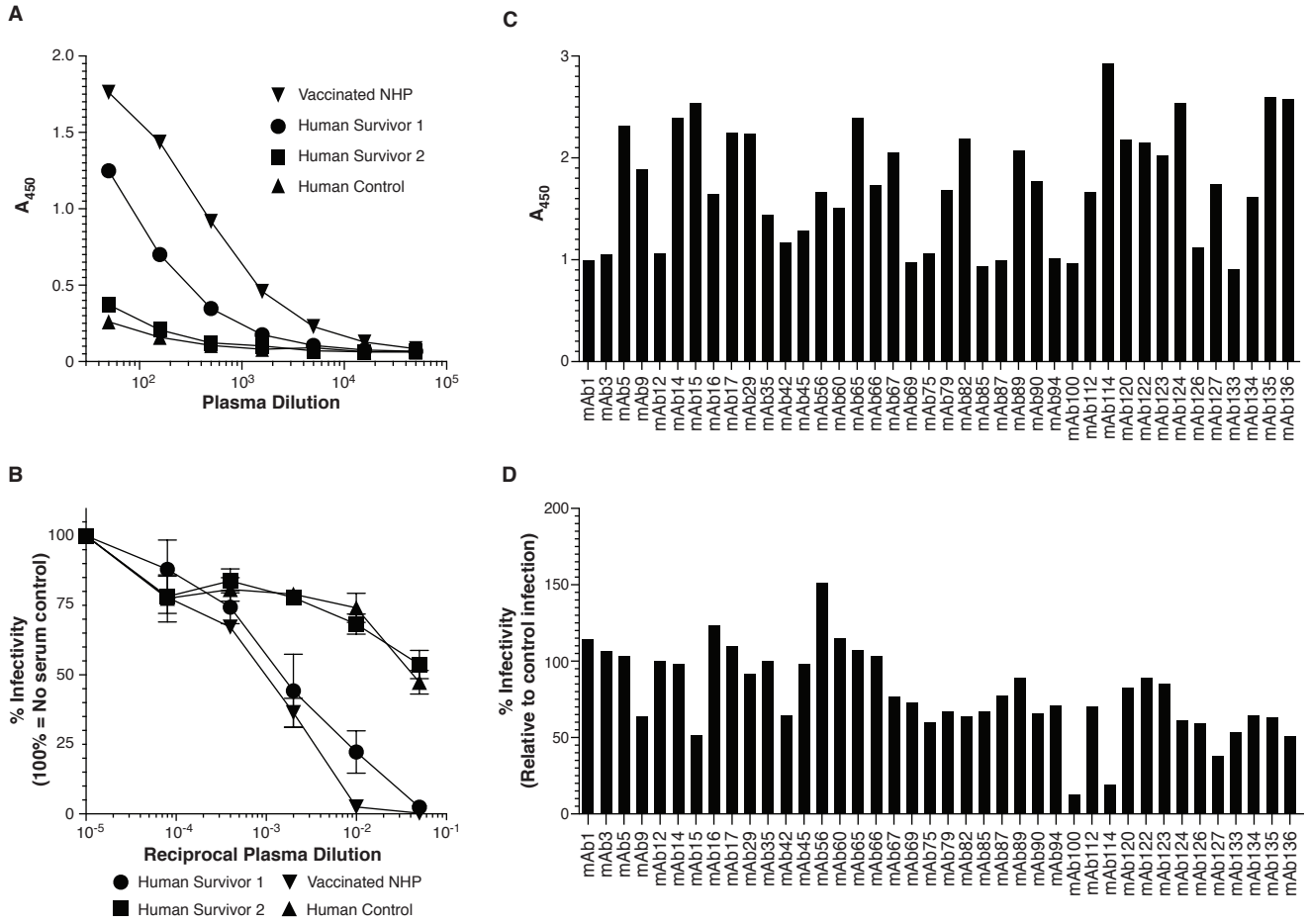

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Figure 1. Isolation of antigen-specific monoclonal antibodies from Ebola virus disease survivor. (A) Plasma obtained from two human survivors, an uninfected human donor and a non-human primate (NHP) vaccinated against EBOV GP were serially diluted and analyzed by GP ELISA, A_{450} (n=1). **(B)** Lentivirus particles expressing luciferase and bearing EBOV GP were incubated in the presence of heat inactivated serum for 1 hour prior to addition to HEK293T. Infection was determined by measuring relative luminescence (RLU) after 3 days. Infection % = (RLU with serum / RLU without serum) X 100% (n=3). **(C)** Immortalized B cell supernatants isolated from Survivor 1 were screened by EBOV GP ELISA A_{450} (n=1). **(D)** Immortalized B cell supernatants from (**C**) were diluted 1:50, incubated with Lentivirus particles pseudotyped with EBOV GP and infection determined as in **(B).** Infection % = (RLU with supernatant / RLU without supernatant) X 100% (n=1).

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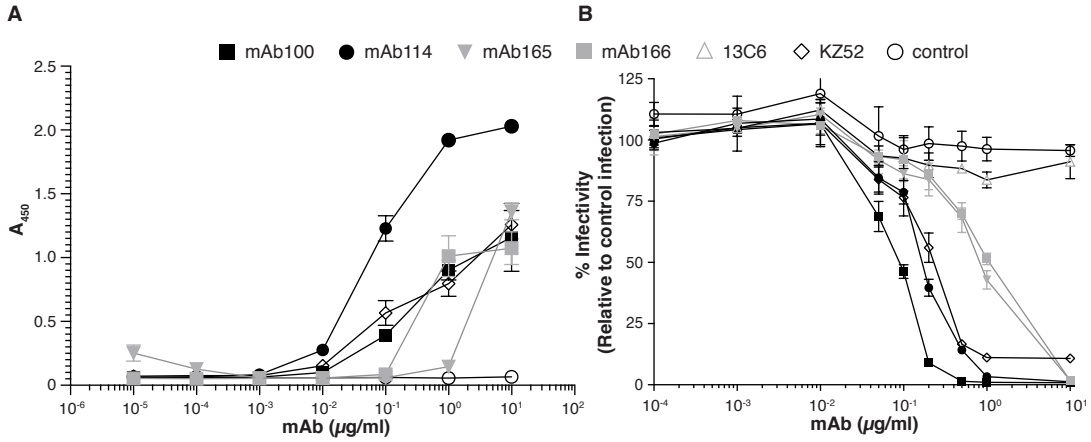
Figure 2



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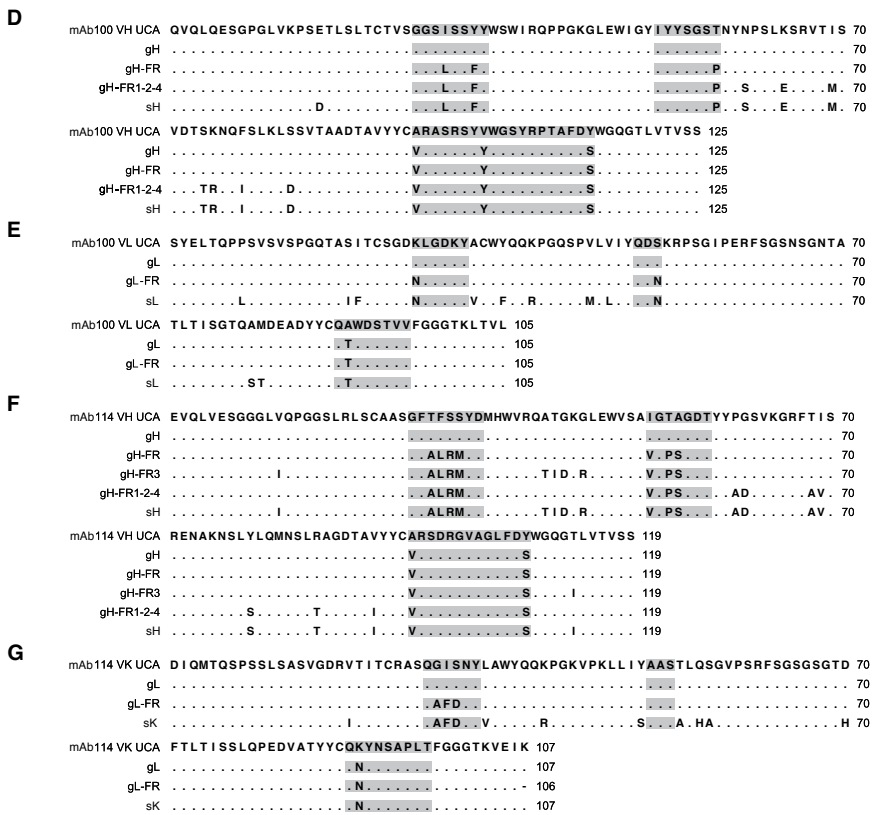
	VH	Identity %	D	JH	VL	Identity %	JL	HCDR3 length	IgG subclass
mAb100	V4-59*01	94.74%	D3-16*02	J4*02	VL3-1*01	92.11%	J2*01	19	IgG3
mAb114	V3-13*01	87.37%	D6-19*01	J4*02	VK1-27*01	92.83%	J4*01	13	IgG1
mAb165	V3-7*01	89.93%	D2-2*02	J4*02	VK1-39*01	89.96%	J1*01	21	IgG1
mAb166	V1-69*04	92.01%	D3-22*01	J6*03	VK3-20*01	97.16%	J3*01	16	IgG3



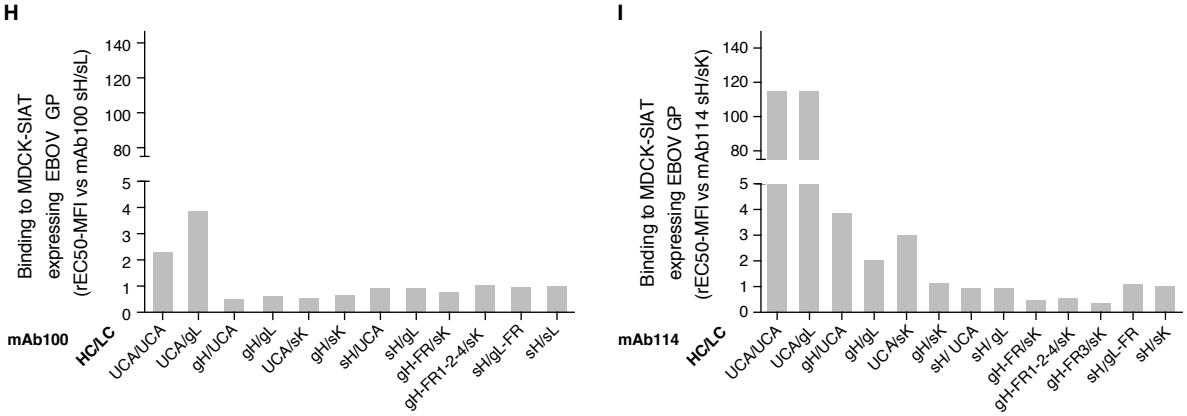


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Figure 2. Characterization of purified EBOV GP monoclonal antibodies. (A) EBOV GP ELISA in the presence of purified monoclonal antibodies as indicated, A_{450}. **(B)** Lentivirus particles pseudotyped EBOV GP particles were incubated with increasing amounts of purified monoclonal antibodies and infection measured as in Fig. 1B. Infection % = (RLU with antibody / RLU without antibody) X 100% (n=3). **(C)** V gene usage, sequence analysis and IgG subclass of antibodies from Survivor 1. **(D) – (G)** Amino acid sequence of mAb100, mAb114 and variants descended from a putative unmutated common ancestor (UCA) for heavy and light chains. Shaded regions represent complementary determination regions 1-3. **(H)** and **(I)** Binding to EBOV GP expressed on the surface of MDCK-SIAT cells by different mAb100 **(H)** and mAb114 **(I)** versions in which all or subsets of somatic mutations in the wild type sH, sL (mAb100) or sK (mAb114) chain were reverted to the germline sequence. Shown is the ratio between the EC_{50} values of the variants and EC_{50} values of the wild-type sH/sL (mAb100) or sH/sK (mAb114). UCA, unmutated common ancestor; gH or gL, germline V-gene revertants of sH, sL, or sK in which the HCDR or LCDR3 are mature; gH-FR or gL-FR, germline V-gene revertants of sH, sL or sK in which the HCDRs or LCDRs are mature; gH-FR1-2-4, germline V-gene revertants of sH in which the HCDRs and HFR3 are mature; gH-FR3, germline V-gene revertants of sH in which the HCDRs and HFR1, HFR2 and HFR4 are mature; wild type, somatically mutated are sH, sL, or sK. EC_{50} ratio values above 100 indicate lack of detectable binding.

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Figure 3

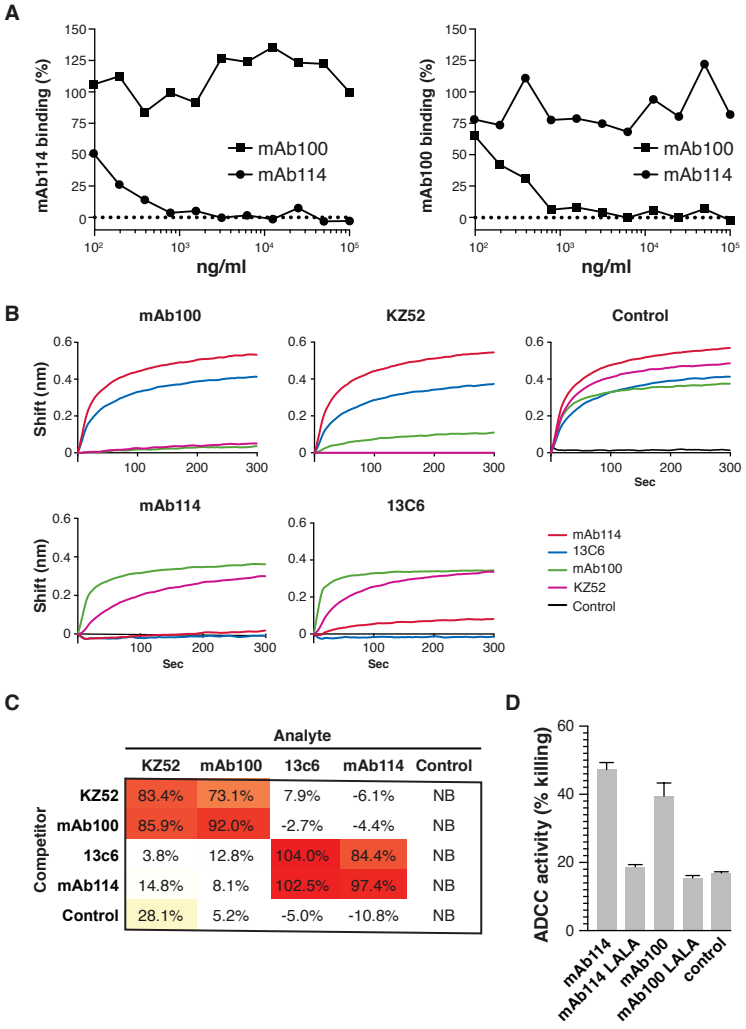

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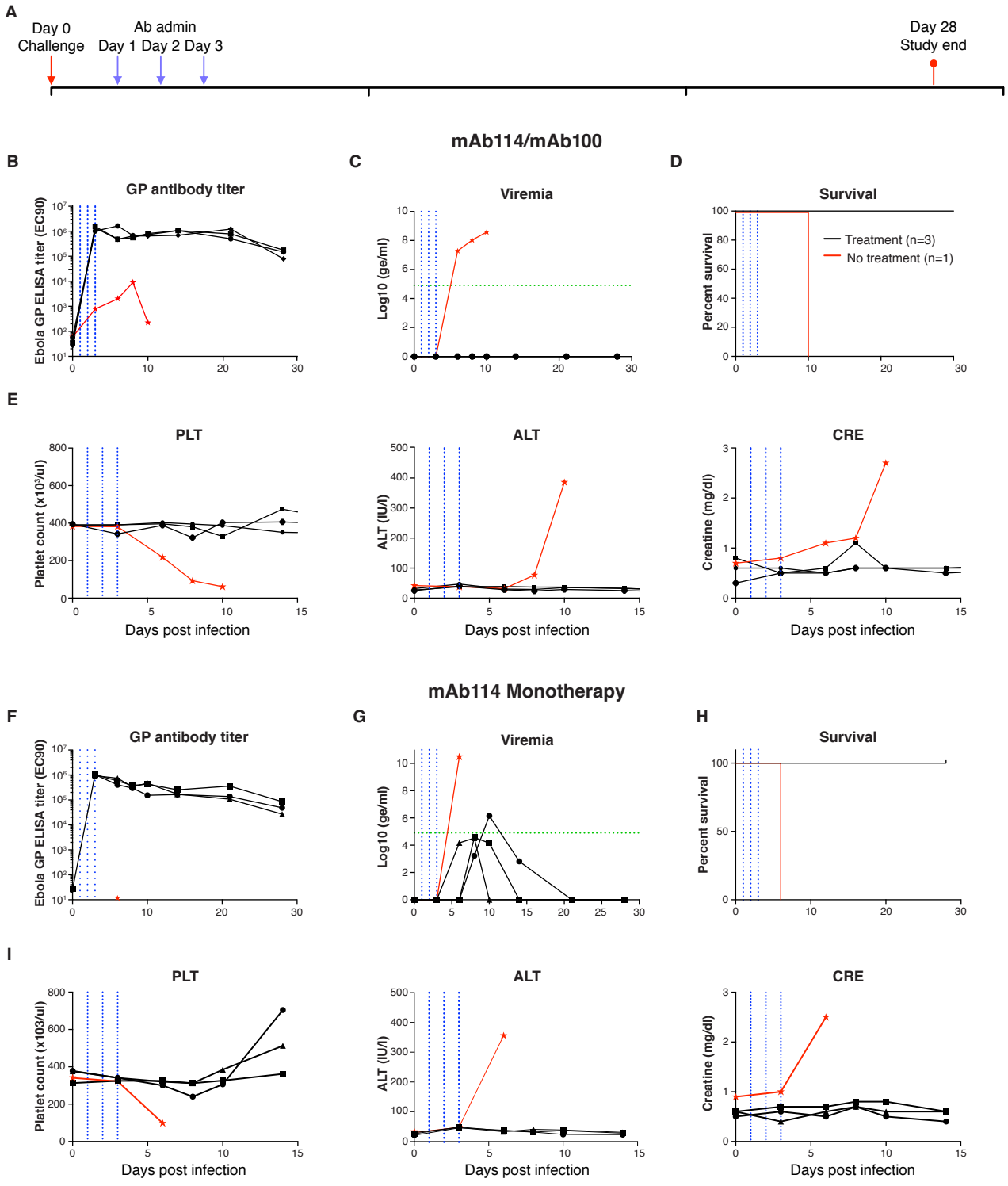
Figure 3. Binding region and effector function. **(A)** Inhibition of binding of biotinylated mAb114 (left) and mAb100 (right) to GP-expressing MDCK-SIAT cells by pre-incubation with increasing amounts of homologous or heterologous unlabeled antibodies. Shown is the percentage of binding of biotinylated antibodies as measured by flow cytometry using fluorophore-conjugated streptavidin. **(B)** and **(C)** Biolayer interferometry competitive binding assay to soluble EBOV GP using mAb100, mAb114, KZ52, 13C6 and isotype negative control. Biosensors were preloaded with GP followed by the competitor and analyte antibodies as indicated. Analyte binding curves (**B**) and quantitated % inhibition (**C**) are reported (n=3). **(D)** Antibody-dependent cell-mediated cytotoxicity (ADCC) assay was determined at 31.6 ng/mL of mAb100, mAb114 (n=3), control antibody or derivative antibodies with LALA mutations that abrogate Fc-mediated killing (n=1).

Figure 4


A
Day 0
Challenge
Ab admin
Day 1 Day 2 Day 3
Day 28
Study end
mAb114/mAb100
B
GP antibody titer
Ebola GP ELISA titer (EC90)
C
Viremia
Log10 (ge/ml)
D
Survival
Percent survival
Treatment (n=3)
No treatment (n=1)
E
PLT
Platlet count (x10³/ul)
ALT
ALT (IU/l)
CRE
Creatine (mg/dl)
Days post infection
mAb114 Monotherapy
F
GP antibody titer
Ebola GP ELISA titer (EC90)
G
Viremia
Log10 (ge/ml)
H
Survival
Percent survival
I
PLT
Platlet count (x103/ul)
ALT
ALT (IU/l)
CRE
Creatine (mg/dl)
Days post infection

368 **Figure 4. Passive transfer of antibody cocktail. (A)** Experimental challenge. Animals
369 were challenged with a lethal dose of EBOV GP on Day 0 and given injections of
370 antibody totaling 50 mg/kg at 24, 48 and 72 hours post-exposure. Surviving animals
371 were euthanized at the conclusion of the study (Day 28). Challenge data from monoclonal
372 antibody mAb114/mAb100 mixture **(B) – (E),** or mAb114 monotherapy **(F)** – **(I)**.
373 Treatment animal in black, untreated control in red. **(B)** and **(F)** Ebola GP specific ELISA
374 titer (EC_{90}). **(C)** and **(G)** Viremia in blood by qRT-PCR expressed as genome equivalents
375 (ge) per mL. **(D)** and **(H)** Survival. **(E)** and **(I)** Selected hematologic and chemistry data.
376 Platelets (PLT), alanine transaminase (ALT), creatinine (CRE).
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Figure 1

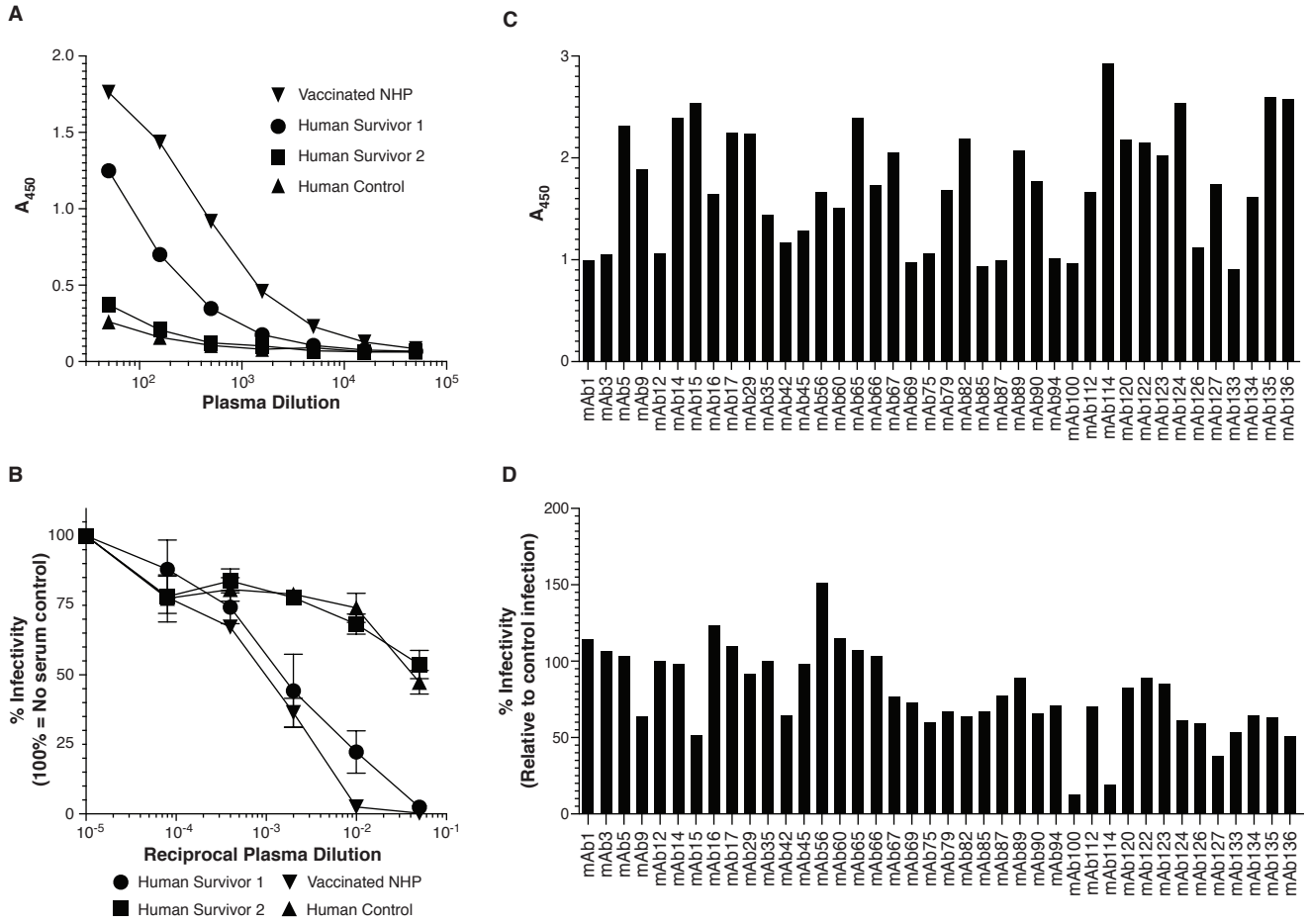

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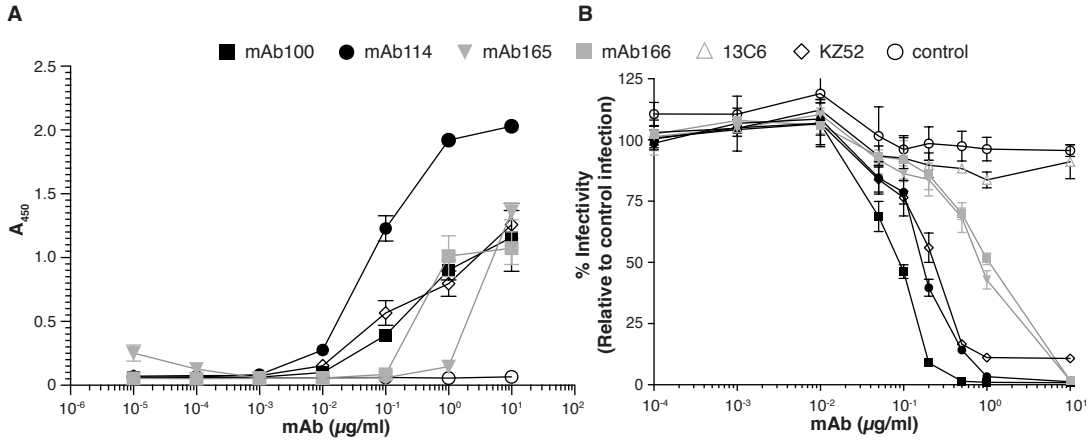
Figure 2



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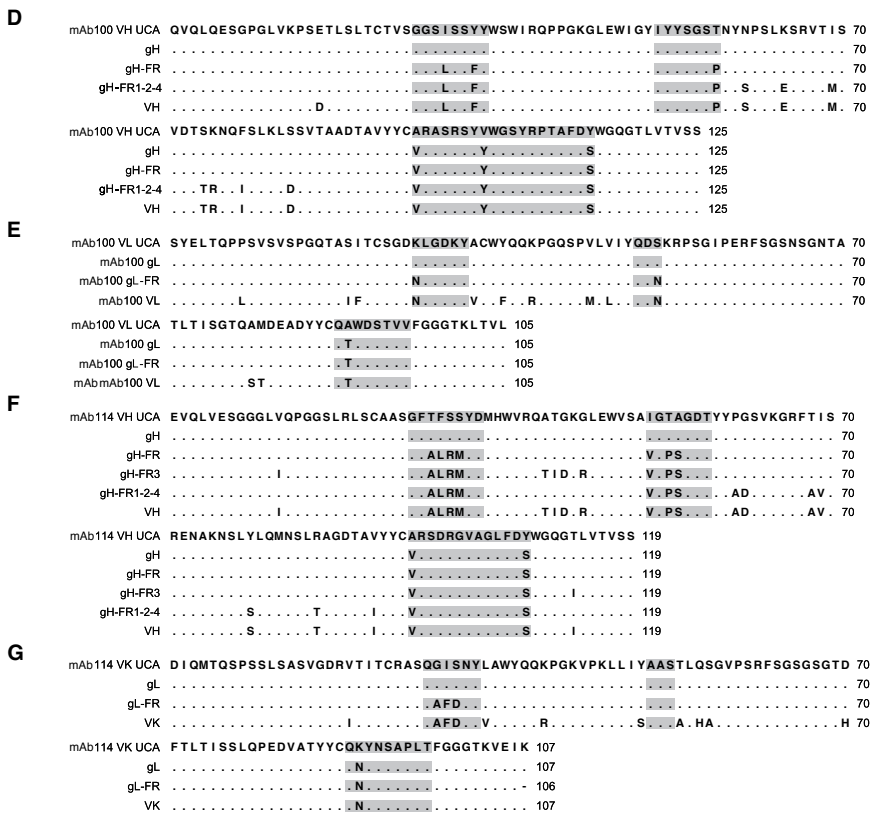
	VH	Identity %	D	JH	VL	Identity %	JL	HCDR3 length	IgG subclass
mAb100	V4-59*01	94.74%	D3-16*02	J4*02	VL3-1*01	92.11%	J2*01	19	IgG3
mAb114	V3-13*01	87.37%	D6-19*01	J4*02	VK1-27*01	92.83%	J4*01	13	IgG1
mAb165	V3-7*01	89.93%	D2-2*02	J4*02	VK1-39*01	89.96%	J1*01	21	IgG1
mAb166	V1-69*04	92.01%	D3-22*01	J6*03	VK3-20*01	97.16%	J3*01	16	IgG3



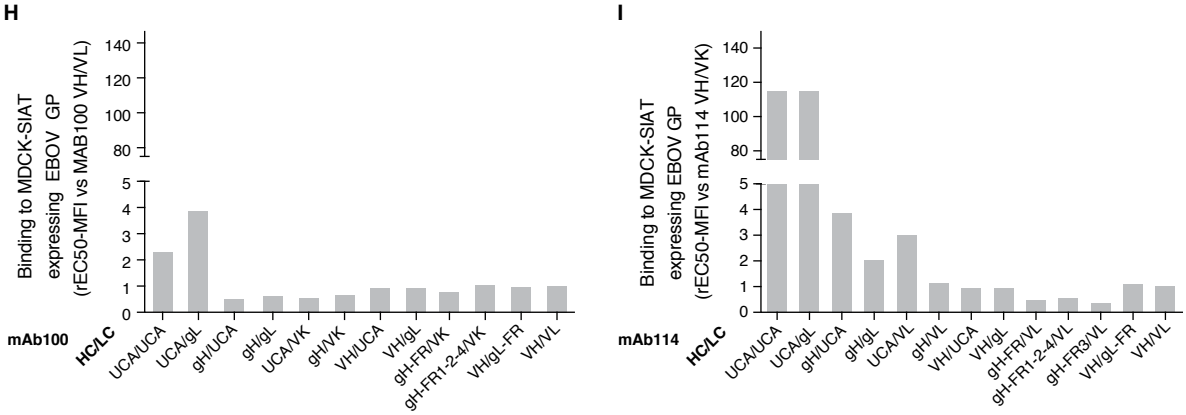


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Figure 3

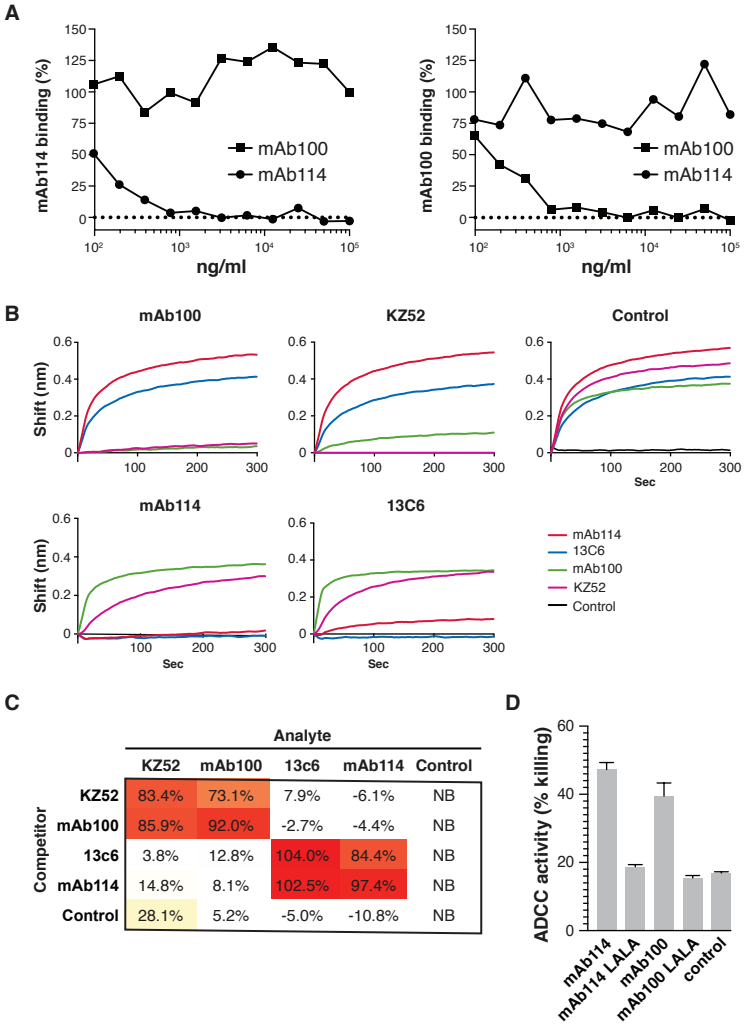

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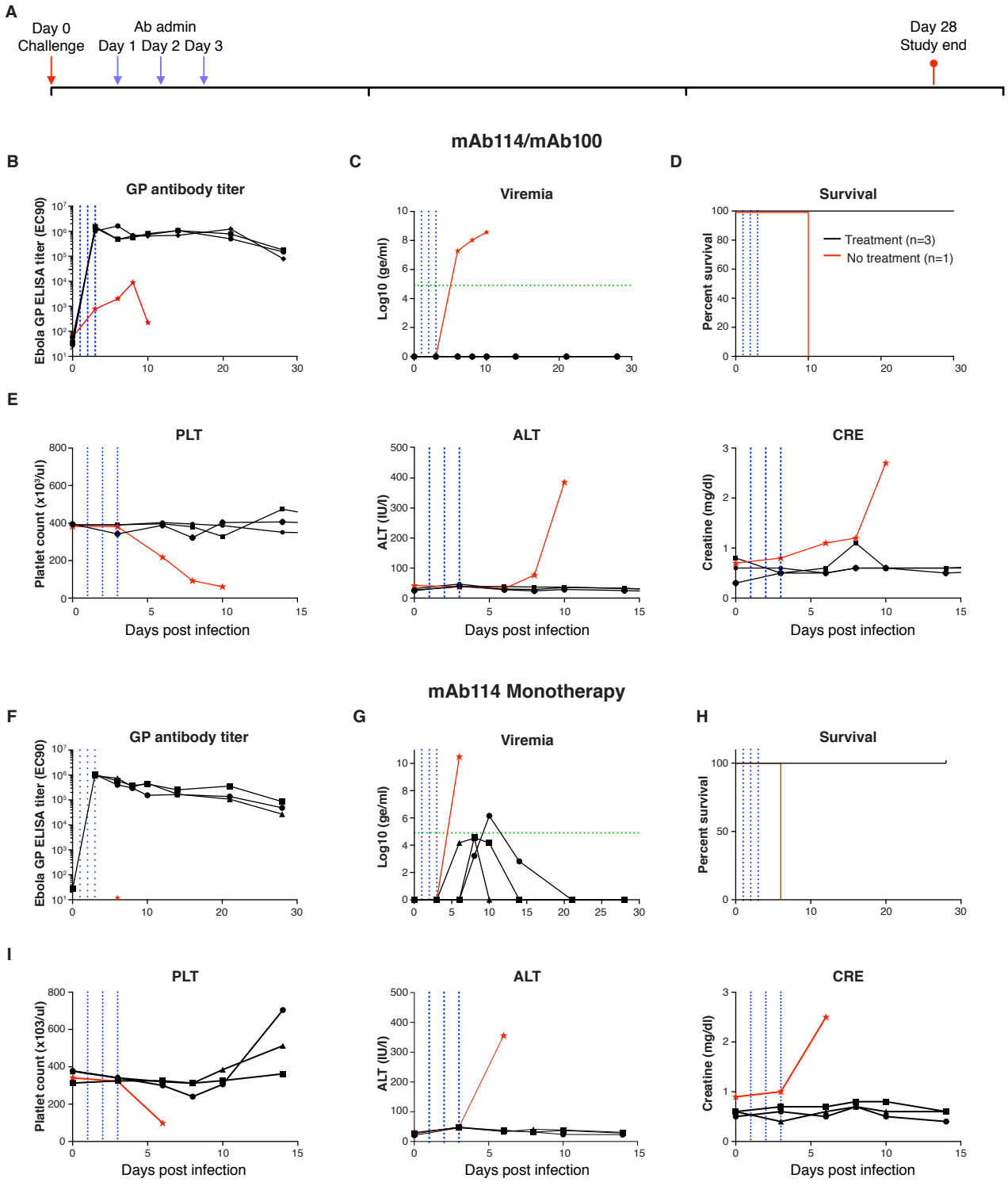
Figure 4


A
Day 0
Challenge
Ab admin
Day 1 Day 2 Day 3
Day 28
Study end
mAb114/mAb100
B
GP antibody titer
Ebola GP ELISA titer (EC90)
C
Viremia
Log10 (ge/ml)
D
Survival
Percent survival
Treatment (n=3)
No treatment (n=1)
E
PLT
Platlet count (x10³/ul)
ALT
ALT (IU/l)
CRE
Creatine (mg/dl)
Days post infection
mAb114 Monotherapy
F
GP antibody titer
Ebola GP ELISA titer (EC90)
G
Viremia
Log10 (ge/ml)
H
Survival
Percent survival
I
PLT
Platlet count (x103/ul)
ALT
ALT (IU/l)
CRE
Creatine (mg/dl)
Days post infection

DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

Figure S1

	Abs 450	
Cell line ID	**Und.**	**1/27**
mAb151	1.119	0.162
mAb152	0.672	0.106
mAb153	0.854	0.131
mAb154	2.361	0.95
mAb155	0.115	0.08
mAb156	1.111	0.161
mAb157	2.256	0.554
mAb158	0.074	0.075
mAb159	3.298	2.529
mAb160	1.493	0.489
mAb161	0.227	0.086
mAb162	0.083	0.074
mAb163	3.099	1.805
mAb164	0.076	0.069
mAb165	3.171	1.722
mAb166	2.894	2.4
mAb167	3.368	2.974
mAb168	0.507	0.114
mAb169	0.081	0.072
mAb170	0.95	0.165
mAb171	1.998	0.895

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Figure S2

mAb	IC50 (μg/mL)	95% CI	IC90 (μg/mL)	95% CI	IC99 (μg/mL)	95% CI	n
KZ52	0.06	0.02 to 0.14	17.21	8.47 to 35.00	>>1000	54,868	6
mAb 100	0.06	0.05 to 0.08	0.61	0.39 to 0.93	7.58	2.999 to 19.16	6
mAb 114	0.09	0.07 to 0.11	0.71	0.44 to 1.16	7.19	2.588 to 19.96	6
mAb 166	0.86	0.72 to 1.02	6.84	4.78 to 9.80	97.25	31.32 to 138.2	4
mAb 165	1.77	1.43 to 2.18	19.46	13.23 to 28.61	267.00	124.9 to 570.8	4

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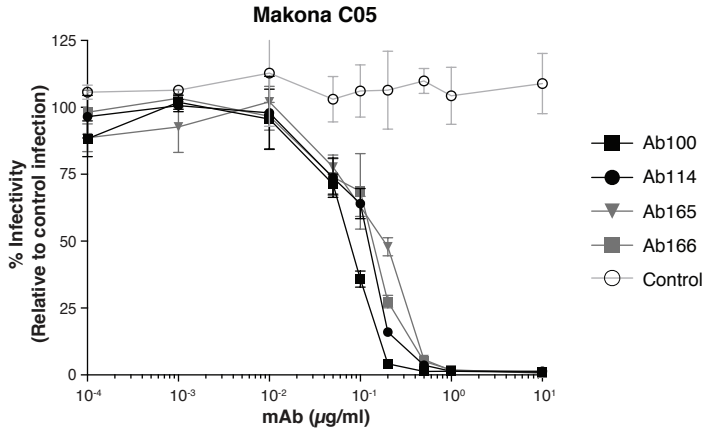
DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

Figure S3



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DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

Figure S4

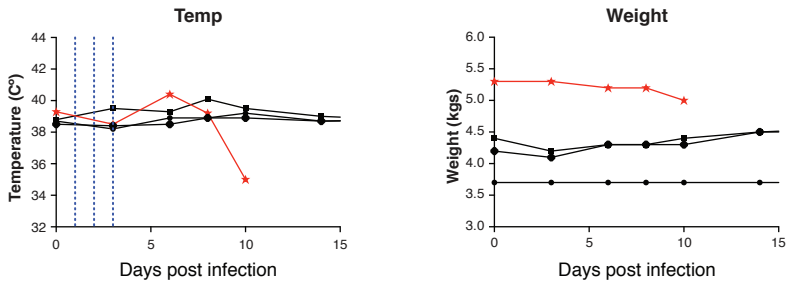

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Figure S5

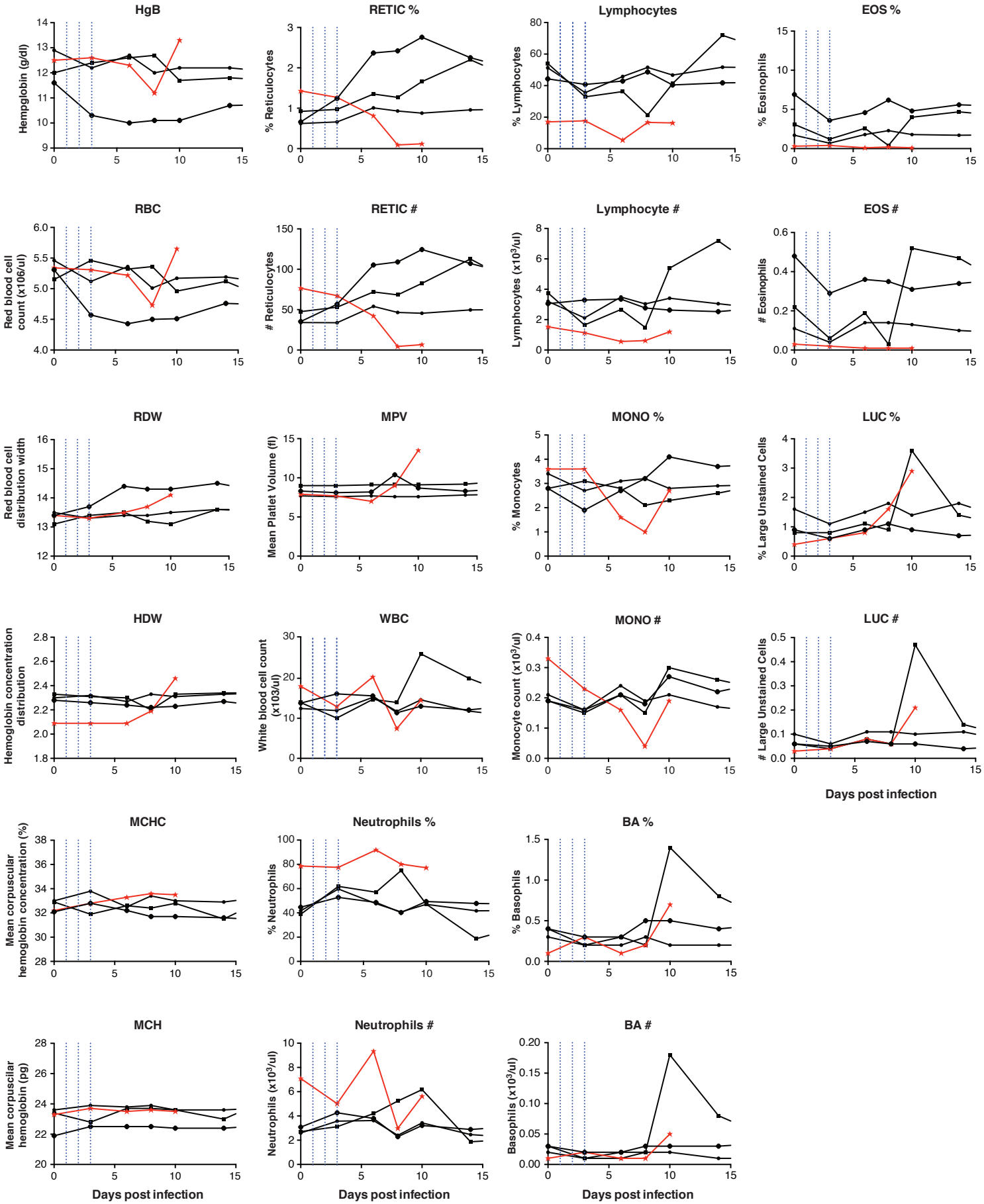

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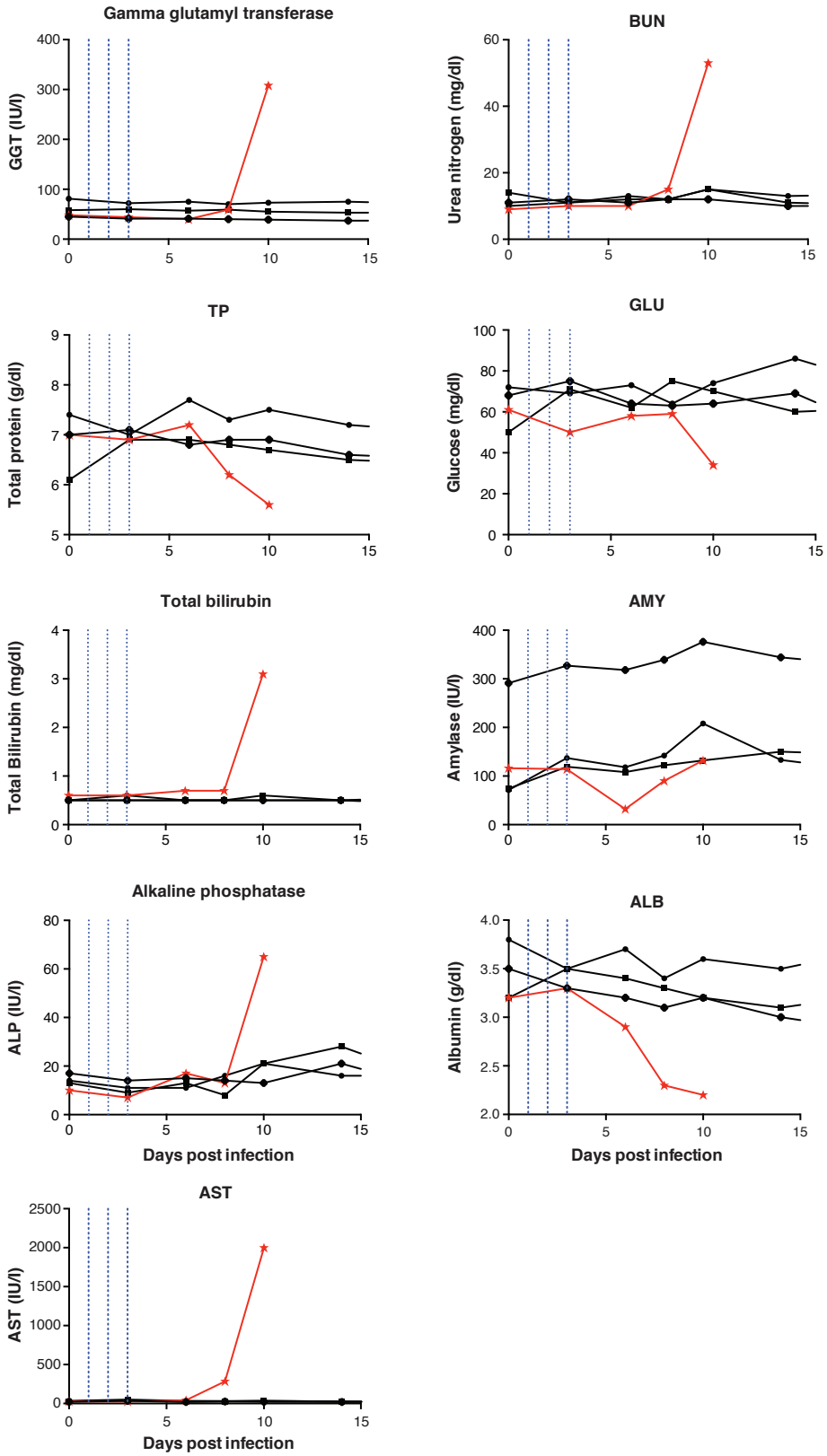
Figure S6


Gamma glutamyl transferase
GGT (IU/l)
BUN
Urea nitrogen (mg/dl)
TP
Total protein (g/dl)
GLU
Glucose (mg/dl)
Total bilirubin
Total Bilirubin (mg/dl)
AMY
Amylase (IU/l)
Alkaline phosphatase
ALP (IU/l)
ALB
Albumin (g/dl)
AST
AST (IU/l)
Days post infection
Days post infection
Days post infection

DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

Figure S7

Cage 1

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	3	0	0	NE	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 2

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	1	2	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 3

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 4

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	2	2																		
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0																		
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y																		
Condition of Stool	0	0	0	0	0	0	0	3	0	3	3																		
Cough	N	N	N	N	N	N	N	N	N	N	N																		
Facial Edema	N	N	N	N	N	N	N	N	N	N	N																		
Rash	0	0	0	0	0	0	0	0	1	3	2																		
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	1	1	0	1	1	0	0																		
Bleeding	N	N	N	N	N	N	N	N	N	N	N																		
Motor Funtion	0	0	0	0	0	0	0	0	0	0	2																		

Observations

Biscuit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Fruit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Urine Output	Y=Yes, N=No, NE=Not Evaluated
Condition of Stool	0=normal, 1=not fully formed, 2=liquid, 3=none, NE=Not Evaluated
Cough	Y=Yes, N=No
Facial Edema	Y=Yes, N=No
Rash	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Rash/ Hemorrhage at Venipunture Site	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Bleeding	N=None, F=Facial, V=Vaginal, O=Other, NE=Not Evaluated
Motor Funtion	0=normal, 1=mild coordination, dysfunction trouble manipulating items, 2=moderate dysfunction trouble grasping, moving, 3=severe dysfunction (uncoordinated actions), NE=Not Evaluated

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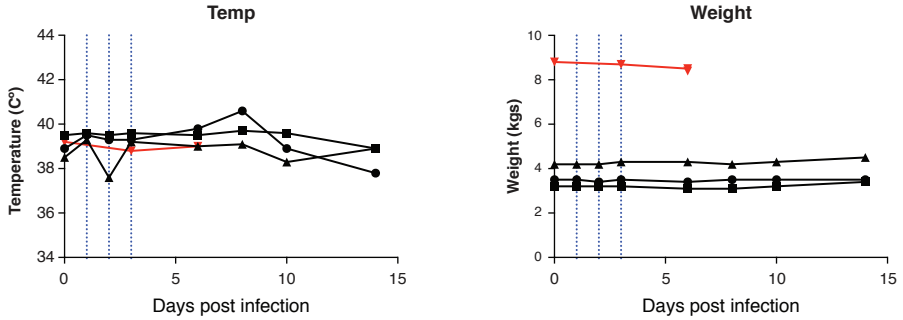
Figure S8


Temp
Temperature (C°)
44
42
40
38
36
34
0
5
10
15
Days post infection
Weight
Weight (kgs)
10
8
6
4
2
0
0
5
10
15
Days post infection

DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

Figure S9

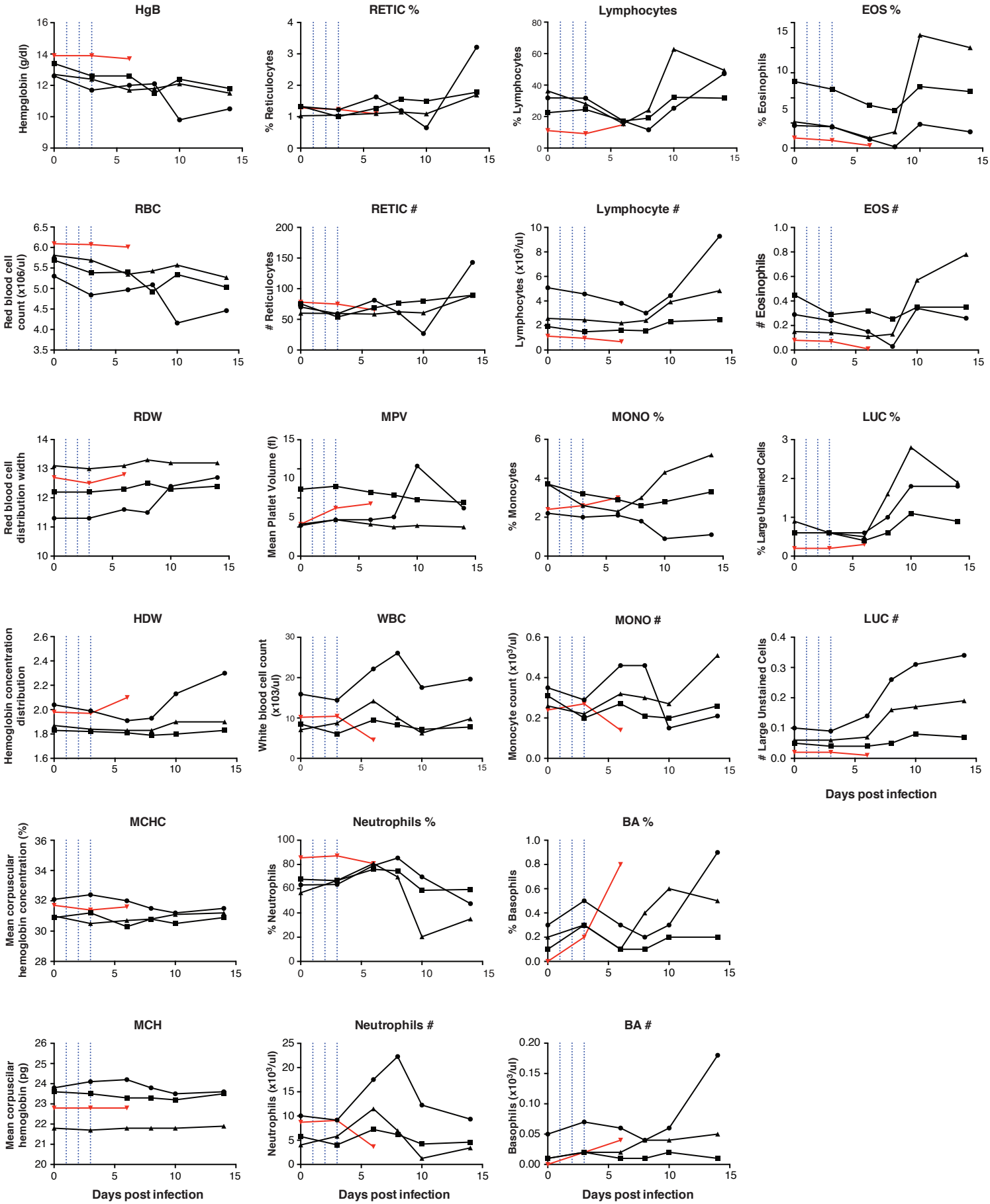


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Figure S10

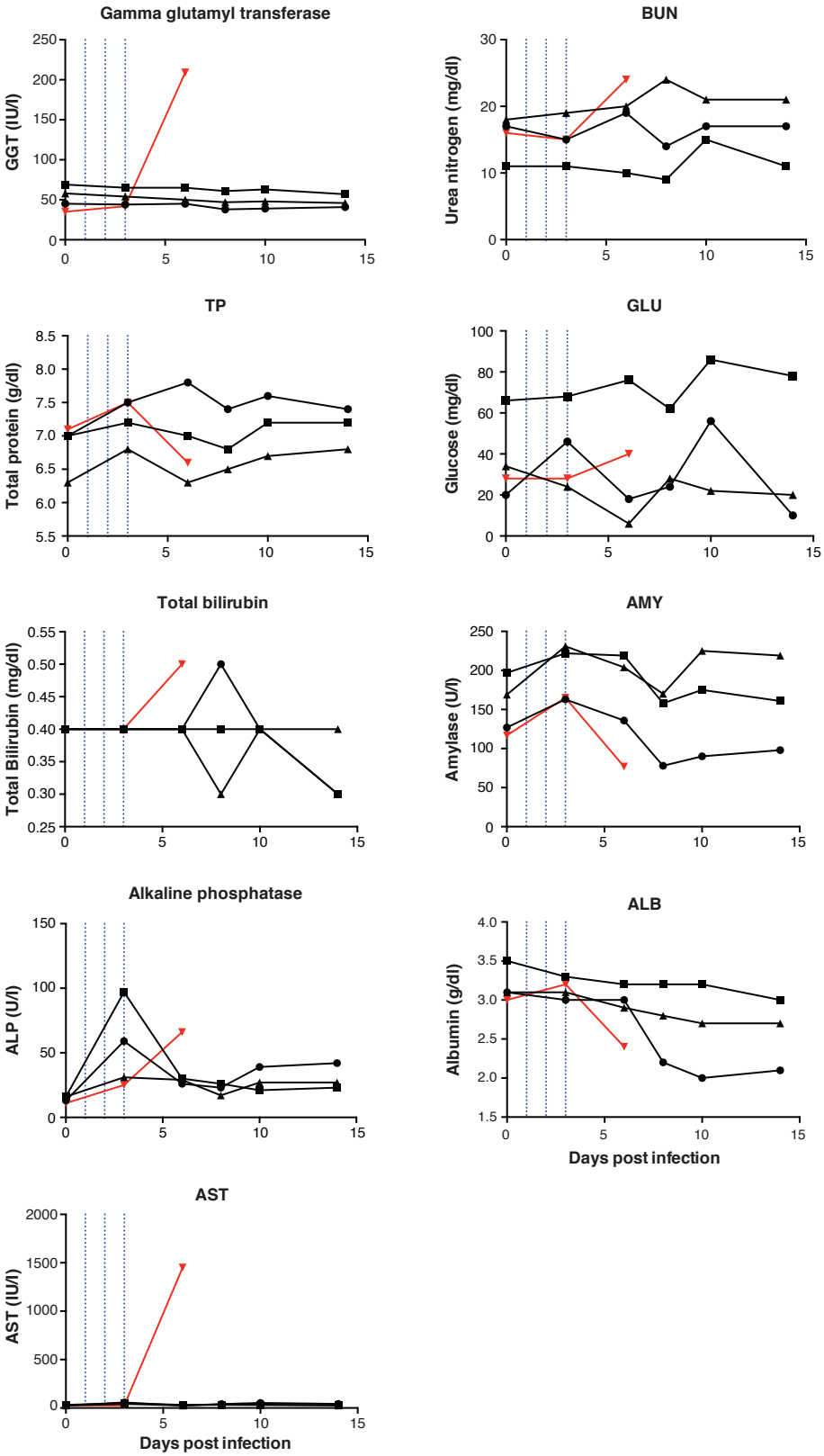
Gamma glutamyl transferase
GGT (IU/l)
BUN
Urea nitrogen (mg/dl)
TP
Total protein (g/dl)
GLU
Glucose (mg/dl)
Total bilirubin
Total Bilirubin (mg/dl)
AMY
Amylase (U/l)
Alkaline phosphatase
ALP (U/l)
ALB
Albumin (g/dl)
AST
AST (IU/l)
Days post infection
Days post infection

Figure S11

Cage 1

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	1	1	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	V	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 2

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	O	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 3

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Primary Euthanasia Criteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 4

Day	0	1	2	3	4	5	6
Biscuit Consumption	0	1	0	0	0	0	2
Fruit Consumption	0	0	0	0	0	0	2
Urine Output	Y	Y	Y	Y	Y	Y	N
Condition of Stool	0	0	0	0	0	0	3
Cough	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0

Observations

Biscuit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Fruit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Urine Output	Y=Yes, N=No, NE=Not Evaluated
Condition of Stool	0=normal, 1=not fully formed, 2=liquid, 3=none, NE=Not Evaluated
Cough	Y=Yes, N=No
Facial Edema	Y=Yes, N=No
Rash	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Rash/ Hemorrhage at Venipunture Site	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Bleeding	N=None, F=Facial, V=Vaginal, O=Other, NE=Not Evaluated
Motor Funtion	0=normal, 1=mild coordination, dysfunction trouble manipulating items, 2=moderate dysfunction trouble grasping, moving, 3=severe dysfunction (uncoordinated actions), NE=Not Evaluated

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Protective Monotherapy Against Lethal Ebola Virus Infection by a Potently Neutralizing Antibody

Davide Corti,[1,4*] John Misasi,[2*] Sabue Mulangu,[2] Daphne A. Stanley,[2] Masaru Kanekiyo,[2] Suzanne Wollen,[3] Aurélie Ploquin,[2] Nicole A. Doria-Rose,[2] Ryan P. Staupe,[2] Michael Bailey,[2] Wei Shi,[2] Misook Choe,[2] Hadar Marcus,[2] Emily A. Thompson,[2] Alberto Cagigi,[2] Chiara Silacci,[1] Blanca Fernandez-Rodriguez,[1] Laurent Perez,[1] Federica Sallusto,[1] Fabrizia Vanzetta,[4] Gloria Agatic,[4] Elisabetta Cameroni,[4] Neville Kisalu,[2] Ingelise Gordon,[2] Julie E. Ledgerwood,[2] John R. Mascola,[2] Barney S. Graham,[2] Jean Jacques Muyembe-Tamfun,[5] John C. Trefry,[3†] Antonio Lanzavecchia,[1†] and Nancy J. Sullivan[2*†]

[1]Immune Regulation Unit
Institute for Research in Biomedicine
CH-6500 Bellinzona, Switzerland

[2]Vaccine Research Center
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda, MD 20892 USA

[3]United States Army Medical Research Institute of Infectious Diseases
Fort Detrick, MD 21702 USA

[4]Humabs BioMed SA
6500 Bellinzona, Switzerland

[5]National Institute for Biomedical Research
National Laboratory of Public Health
Kinshasa B.P. 1197, Democratic Republic of the Congo

*These authors contributed equally to this work.
†These authors contributed equally to this work.

Corresponding Author: Nancy J. Sullivan
Vaccine Research Center, National Institutes of Health
40 Convent Drive, Bldg. 40/2509
Bethesda, Maryland 20892, USA
Phone number: 301-435-7853
njsull@mail.nih.gov

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Supplementary Materials:

Materials and Methods

Isolation of monoclonal antibodies from EBOV survivors. Two subjects who survived the 1995 EBOV Kikwit variant outbreak in the Democratic Republic of Congo were identified and enrolled in VRC200 clinical trial #NCT00067054 after giving signed informed consent. Peripheral blood mononuclear cells (PBMCs) were obtained, stained with directly labeled antibodies to CD22 (Pharmingen) and to immunoglobulin IgM, IgD, and IgA. $CD22^{+}IgM^{-}IgD^{-}IgA^{-}$ B cells were isolated using FACS Aria, pulsed with Epstein-Barr Virus (50% B958 supernatant) and seeded at 30 cells/well (for a total of 2.7 $\times 10^{5}$ purified cells) in replicate cultures in medium supplemented with CpG 2006 and irradiated allogeneic PBMCs, as previously described (*9*). Culture supernatants were collected after 2 weeks and tested for binding to ELISA plates coated with EBOV GP (Mayinga variant), their specificity was confirmed using an unrelated antigen (tetanus toxoid) and positive cultures were further tested for their ability to neutralize EBOV pseudoviruses. Cultures that scored positive in the EBOV neutralization assay were sub-cloned by limiting dilution.

Antibody purification, labeling, genetic analysis, and reversion to germline. The usage of VH and VL gene segments was determined by sequencing, and analysis for homology to known human V, D, and J genes was performed using the IMGT database (http://www.imgt.org/). Human antibodies were affinity purified by protein A

chromatography (GE Healthcare) and dialyzed against PBS. Selected antibodies were biotinylated using the EZ-Link NHS-PEO Solid Phase Biotinylation Kit (Pierce). Antibodies were also produced recombinantly by cloning VH and VL genes via PCR into human Igγ1, Igκ (mAb114, 165, 166), and Igλ (mAb100) expression vectors using gene-specific primers (*19*). Antibodies used for animal studies were produced by transient transfection of suspension cultured 293FreeStyle cells (Invitrogen) with PEI or Expi cells with Expifectamine293 (Invitrogen). Supernatants from transfected cells were collected after 6-10 days of culture and IgGs were affinity purified by Protein A chromatography (GE Healthcare) and dialyzed against PBS. Purified mAbs were then concentrated with Amicon Ultra centrifugal filters and sterilized by 0.22 μm filtration. The purity was assessed by SEC-HPLC and SDS-PAGE. Endotoxin content was measured with the Endpoint Chromogenic LAL assay (QCL-1000 TM assay, Lonza) according to manufacturing instructions and shown to be below 0.25 EU/ml. Antibody concentrations were determined using the BCA Protein Assay Kit (Thermo Scientific) using Rituximab (Roche) as internal standard or A_{280} using an Nanodrop (Thermo Scientific). Germlined VH and VL nucleotide sequences were synthesized by Genscript, and their accuracy was confirmed by sequencing.

Antibodies. KZ52 monoclonal antibody used in ELISA assay a kind gift from Dennis Burton. KZ52 used elsewhere and 13C6 was purchased from IBT Bioservices. Unless otherwise noted isotype control antibody was an anti-HIV gp120 IgG1.

Antibody neutralization assay. Supernatants or purified mAbs from immortalized B cell clones isolated from EVD survivor donors were assessed for neutralization potency using a single-round infection assay with EBOV GP-pseudotyped lentiviruses particles which express a luciferase reporter gene following entry (*20*). Unless indicated, all experiments utilized particles bearing GP from the EBOV Mayinga variant. In brief, HEK293T cells were used as infection targets and incubated in a 96-well plate 1 day before infection with pseudovirus in the presence of serially diluted supernatant or purified mAbs. Infected target cells were lysed 72 hours after infection and assayed with the Luciferase Assay System or Bright Glo (Promega), using a Victor X3 Plate Reader (PerkinElmer) to detect luciferase activity.

ELISA for serum antibody titer and GP-binding. Binding of EVD survivor's polyclonal sera, monoclonal antibodies and antibody in non-human primates to EBOV GP was evaluated by enzyme-linked immunosorbent assay (ELISA) as described previously (*20*). Titers for survivor and non-human primates were calculated as reciprocal EC_{90} values (*20*).

Ebola virus GP vectors. Plasmid vector pVR1012 WT GP (Z) has been described previously (*21*). A vector expressing a soluble mucin deleted (ΔMuc) GP, GPΔMucΔTM-GCN4 HisSA (Δ309-505, Δ657-676), was made using codon optimization and then synthesized and directly cloned in frame to a GCN4 trimerization domain-His-Strep Tactin domains (MKQIEDKIEEILSKIYHIENEIARIKKLIGEVASSSIEGRGSHHHHHHHSAWSHPQFE

K) and sequence verified by Genscript. EBOV GP variant Makona-C05 (Acc#KJ660348) was codon optimized, synthesized and sequence verified by Genscript.

Antibody-dependent cell-mediated cytotoxicity (ADCC). rAd5 EBOV GP-transduced and non-transduced HEK293T cells were double labeled with membrane-bound and intracellular stains in order to detect ADCC activity. Cells were incubated with 8 μM Plum stain (Plum cell labeling kit M.T.T.I. CellVue) followed by FBS. The cells were then washed with RPMI 1640, incubated with 5 μM Carboxyflourescein Succinimidyl ester (CFSE) (Vybrant CFDA SE cell tracer kit, Invitrogen), incubated with FBS and washed again with RPMI 1640. Doubly labeled EBOV GP expressing cells were plated in a V-bottomed 96-well plate at 5,000 cells/well. Antibodies were added to duplicate samples at 31.6 ng/ml to the target cells for 20 minutes at room temperature. RSV antibody (palivizumab) was used as a control antibody. Effector cells resuspended in RPMI were then added to the target cells at the effector-to-target cell (ET) ratio 1:50 which was found to give the best signal to noise ratio. Each plate was incubated for 4 hr at 37°C/5% CO_2. After 4 hr, plates were centrifuged at 250 x g and cells were fixed with 1% Paraformaldehyde (PFA) and analyzed via flow cytometry. As a control, labeled non-transduced HEK293T cells were also used as targets for ADCC activity. Thirty thousand non-gated events were acquired within 6 hr after the ADCC assay using an LSRII cytometer (Becton Dickinson). The CFSE emission channel was read in B515 using a neutral density filter and Plum emission was read in R660. Following acquisition, analysis was performed using FlowJo software (Tree Star). Percent killing was obtained by quantifying dead cells (Plum+,$CFSE^-$) out of the total Plum positive population. For

mAbs, ADCC killing was measured by subtracting percent killing of nontransduced cells from percent killing of transduced cells.

Antibody variants. UCA sequences of the isolated antibodies were determined with reference to the IMGT database (http://www.imgt.org/). Antibody variants in which single or multiple mutations were reverted to the germline sequence were produced by gene synthesis (Genscript) and used to produce a large set of mAb114 and mAb100 antibody variants.

Binding of antibody variants to transfected cells. mAb114 and mAb100 antibody variants were used to stain MDCK-SIAT1 cell lines transduced to express EBOV GP as a stable membrane protein (Makona variant). Binding of antibodies was analysed using a Becton Dickinson FACS Canto2 (BD Biosciences) with FlowJo software (TreeStar). The relative affinities of antibody binding to surface GP were determined by interpolating the concentration of antibody required to achieve 50% maximal binding (EC_{50}) from the plotted binding curves using the mean-fluorescence intensity (MFI) fitted with a 4-parameter nonlinear regression with a variable slope.

Inhibition of binding assay on GP-expressing cells. mAb100 and mAb114 were biotinylated using the EZ-Link NHS-PEO solid phase biotinylation kit (Pierce). Labeled antibodies were tested for binding to GP-expressing MDCK-SIAT-1 cells to determine the optimal concentration of each antibody to achieve 70–80% maximal binding. The biotin-labelled antibodies were then used as probes to assess, by flow cytometry, whether

158 their binding (measured using fluorophore-conjugated streptavidin) was inhibited by pre-
159 incubation of GP cells with homologous or heterologous unlabelled antibodies.
160
161 **Production of purified GP.** Expi (Invitrogen) cells were transfected with GP
162 ΔMucΔTM-GCN4 HisSA and pCMV-Sport Furin (7:3 ratio) using 293Fection
163 (Invitrogen) at a ratio of 2 mL 293Fectin:1mg total DNA. 18-24 hours following
164 transfection, 1/10th volume of AbBooster (ABI Scientific) was added and culture media
165 collected 5 days later. Supernatant was filtered and protein purified as described
166 previously (*22*).
167
168 **Biolayer interferometry antibody cross-competition assay**. Antibody cross-
169 competition was determined based on biolayer interferometry using a fortéBio Octet
170 HTX instrument. EBOV GP ΔMuc protein was loaded onto HIS biosensors (AR2G,
171 fortéBio) through amine coupling for 600 s. Biosensors were equilibrated for 120 s in
172 1% BSA in PBS (BSA-PBS) prior to capturing competitor mAbs. GP proteins were
173 diluted to 10 μg/mL; mAbs KZ52, mAb100, mAb114, 13C6, and IgG1 isotype control
174 Ab were diluted to 35 μg/mL in BSA-PBS. Binding of competitor mAbs was assessed
175 for 300 s followed by a brief equilibration for 60 s prior to binding assessment of probing
176 mAbs. Binding of probing mAbs was assessed for 300 s. Percent inhibition (PI) of
177 probing mAbs binding to GP by competitor mAbs was carried out by an equation: PI =
178 100 − [(probing mAb binding in the presence competitor mAb) ⁄ (probing mAb binding
179 in the absence of competitor mAb)] × 100. All the assays were performed in duplicate
180 and with agitation set to 1,000 rpm at 30°C.

Animal study and safety. Research was conducted under an IACUC-approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facilities where this research was conducted are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adhere to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011. Animal study protocols were approved by both the Vaccine Research Center and United States Army Medical Research Institute of Infectious Diseases IACUCs. All animals were Vietnamese-origin rhesus macaques (*Macaca mulatta*), female, approximately 2–5 years of age and were obtained from Covance. Animals were randomly assigned to treatment groups based on sequential selection from a population inventory. Sample sizes of three animals per BSL4 EBOV challenge group provide 80% power to detect a difference in survival rates assuming 100% survival (3/3 treated survive) vs. 0% survival in negative controls at the 95% confidence level (1-tailed Fisher exact test). Prior to blood sampling or treatment, animals were anesthetized with ketamine or telazol.

Antibody administration. In the mAb100/mAb114 cocktail challenge, antibodies were mixed in PBS at 4 mg/mL of mAb100 and 46 mg/mL of mAb114 for a total antibody concentration of 50 mg/mL. In the second challenge, animals received 50 mg/mL of mAb114 in PBS. Antibodies were administered via intravenous injection in peripheral veins using ≤20 gauge butterfly needles over a period ≥ 15 minutes in a single bolus via syringe pump.

EBOV challenge. Animal studies conducted at USAMRIID were approved by the IACUC. Animals were transferred one week prior to challenge to the Bio-Safety Level-4 (BSL-4) facility for exposure to a lethal (1000 PFU) i.m. EBOV Kikwit variant challenge. Challenge studies included a single unvaccinated animal (control); the use of historical control (n>50) allows for one untreated control to be used in each challenge experiment. While at USAMRIID the monkeys were fed and checked daily. During the EBOV challenge study, blood was collected from the NHP for hematological, biochemical and virological analyses. Following the development of clinical signs, animals were checked multiple times daily. Institute scoring criteria were used to determine timing of humane euthanasia under anesthesia.

Detection of EBOV. RNA was isolated from plasma of EBOV-exposed NHP by real time qPCR as described previously (*23*). EDTA plasma was added to TriReagent LS (Sigma), 1 part to 3 parts, in preparation for qRT-PCR. Inactivated samples were extracted and eluted with AVE Buffer (QIAGEN, Valencia, CA) using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). All samples were run on an Applied Biosystems 7500 Fast Dx Real-Time PCR instrument (Life Technologies, Grand Island, NY). Reactions were performed with SuperScript II One-Step RT-PCR System (Life Technologies, Grand Island, NY) with additional $MgSO_4$ added to a final concentration of 3.0 mM. All samples were run in triplicate 5 µL each. The average of the triplicates was multiplied by 200 to obtain genomes equivalents per mL, then multiplied by a dilution factor of 4 for the final reported value. The sequence of the

primer and probes for the EBOV glycoprotein are described below. The genomic equivalents were determined using a synthetic RNA standard curve of known concentration. Forward primer: 5′ - TTT TCA ATC CTC AAC CGT AAG GC - 3′ ; REVERSE PRIMER : 5′ - CAG TCC GGT CCC AGA ATG TG - 3′ ; PROBE: 6FAM - CAT GTG CCG CCC CAT CGC TGC – TAMRA.

Supplementary Figures

Figure S1-S11

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Figure S1

	Abs 450	
Cell line ID	**Und.**	**1/27**
mAb151	1.119	0.162
mAb152	0.672	0.106
mAb153	0.854	0.131
mAb154	2.361	0.95
mAb155	0.115	0.08
mAb156	1.111	0.161
mAb157	2.256	0.554
mAb158	0.074	0.075
mAb159	3.298	2.529
mAb160	1.493	0.489
mAb161	0.227	0.086
mAb162	0.083	0.074
mAb163	3.099	1.805
mAb164	0.076	0.069
mAb165	3.171	1.722
mAb166	2.894	2.4
mAb167	3.368	2.974
mAb168	0.507	0.114
mAb169	0.081	0.072
mAb170	0.95	0.165
mAb171	1.998	0.895

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Figure S1. Second screening of immortalized memory B-cells from Survivor. 14 million PBMCs were used to isolate 59,500 IgG memory B cells which were immortalized as in Fig. 1A-D. After removal of non-specific binding, 21 culture supernatants were found to specifically bind Ebola GP as measured by ELISA. Shown are ELISA A_{450} values for undiluted and 1:27 dilutions of the supernatants. Amongst the 21 supernatants, only 2 B cell clones (mAb165, mAb166) were rescued for further analysis.

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Figure S2

mAb	IC50 (μg/mL)	95% CI	IC90 (μg/mL)	95% CI	IC99 (μg/mL)	95% CI	n
KZ52	0.06	0.02 to 0.14	17.21	8.47 to 35.00	>>1000	54,868	6
mAb 100	0.06	0.05 to 0.08	0.61	0.39 to 0.93	7.58	2.999 to 19.16	6
mAb 114	0.09	0.07 to 0.11	0.71	0.44 to 1.16	7.19	2.588 to 19.96	6
mAb 166	0.86	0.72 to 1.02	6.84	4.78 to 9.80	97.25	31.32 to 138.2	4
mAb 165	1.77	1.43 to 2.18	19.46	13.23 to 28.61	267.00	124.9 to 570.8	4

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246 **Figure S2. Neutralization of isolated monoclonal antibodies.** Neutralization assays
247 were performed as in Fig. 2B. IC_{50}, IC_{90}, and IC_{99} were determined using non-linear
248 regression-variable slope (Graph Pad).
249
250

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Figure S3

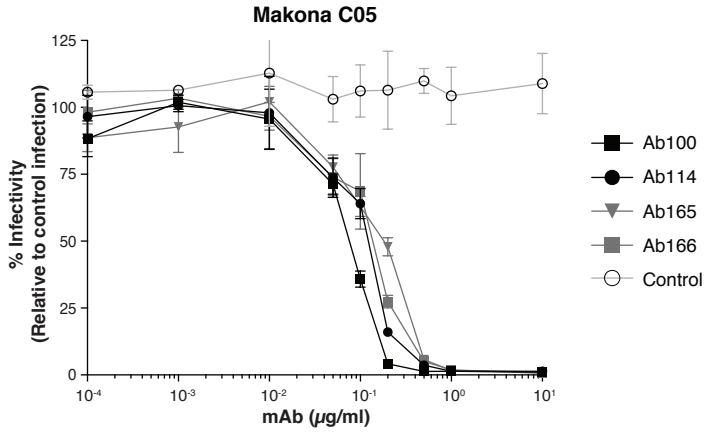

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Figure S3. Inhibition of EBOV Makona variant by mAb100 and mAb114. Lentivirus particles bearing GPs from EBOV Makona variant were incubated with serially diluted mAb100, mAb114 or isotype control. Infection measured as in Fig. 2B (n=3).

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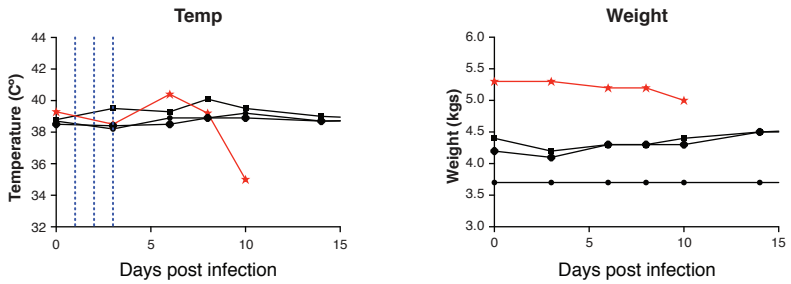
Figure S4



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256 **Figure S4. Additional clinical data from passive transfer of mAb114/mAb100.**

257

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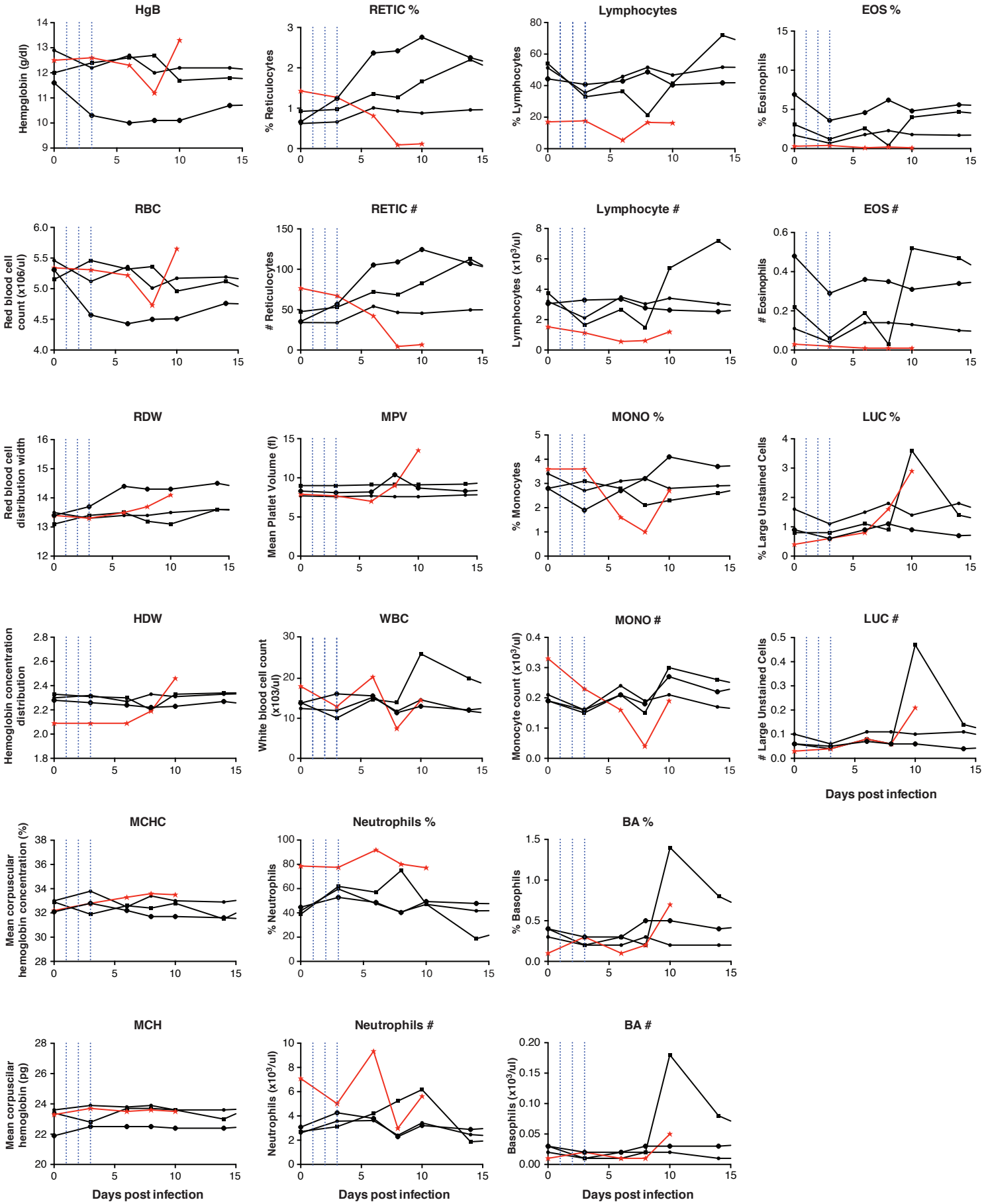
Figure S5



UNCLASSIFIED

259 **Figure S5**. **Additional hematology data from passive transfer of mAb114/mAb100.**

260

261

Figure S6

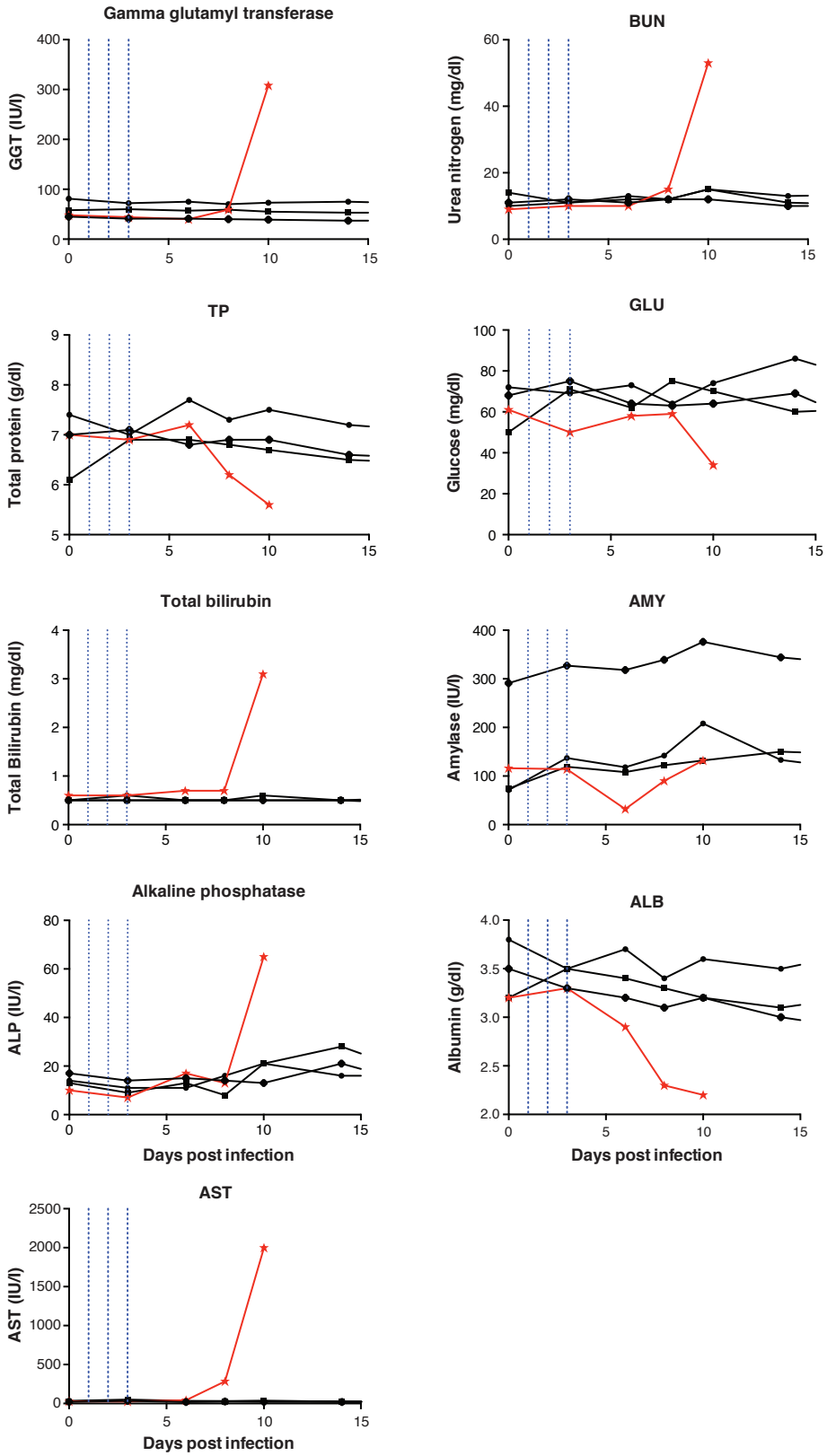

Gamma glutamyl transferase
GGT (IU/l)
BUN
Urea nitrogen (mg/dl)
TP
Total protein (g/dl)
GLU
Glucose (mg/dl)
Total bilirubin
Total Bilirubin (mg/dl)
AMY
Amylase (IU/l)
Alkaline phosphatase
ALP (IU/l)
ALB
Albumin (g/dl)
AST
AST (IU/l)
Days post infection
Days post infection
Days post infection

Figure S6. Additional serum chemistries from passive transfer of mAb114/mAb100.

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Figure S7

Cage 1

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	3	0	0	NE	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 2

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	1	2	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 3

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	NE	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 4

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	2	2																		
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0																		
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y																		
Condition of Stool	0	0	0	0	0	0	0	3	0	3	3																		
Cough	N	N	N	N	N	N	N	N	N	N	N																		
Facial Edema	N	N	N	N	N	N	N	N	N	N	N																		
Rash	0	0	0	0	0	0	0	0	1	3	2																		
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	1	1	0	1	1	0	0																		
Bleeding	N	N	N	N	N	N	N	N	N	N	N																		
Motor Funtion	0	0	0	0	0	0	0	0	0	0	2																		

Observations

Biscuit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Fruit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Urine Output	Y=Yes, N=No, NE=Not Evaluated
Condition of Stool	0=normal, 1=not fully formed, 2=liquid, 3=none, NE=Not Evaluated
Cough	Y=Yes, N=No
Facial Edema	Y=Yes, N=No
Rash	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Rash/ Hemorrhage at Venipunture Site	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Bleeding	N=None, F=Facial, V=Vaginal, O=Other, NE=Not Evaluated
Motor Funtion	0=normal, 1=mild coordination, dysfunction trouble manipulating items, 2=moderate dysfunction trouble grasping, moving, 3=severe dysfunction (uncoordinated actions), NE=Not Evaluated

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Figure S7. Clinical observation scoring from passive transfer of mAb114/mAb100.

Data from multiple observations each day post-exposure are presented as the maximal level observed for that day.

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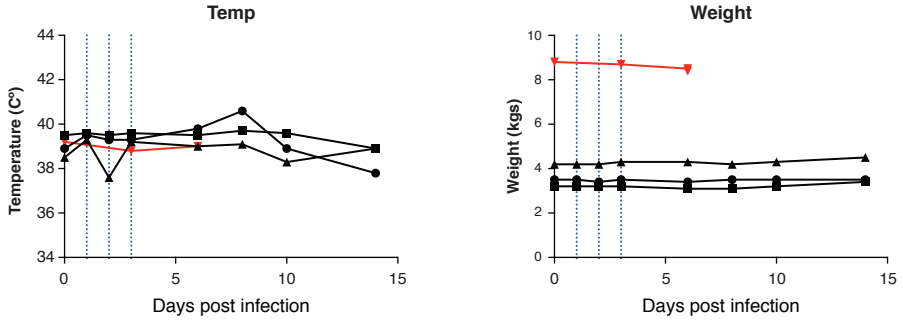
Figure S8


Temp
Temperature (C°)
44
42
40
38
36
34
0
5
10
15
Days post infection
Weight
Weight (kgs)
10
8
6
4
2
0
0
5
10
15
Days post infection

DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

Figure S8. Additional clinical data from passive transfer of mAb114.

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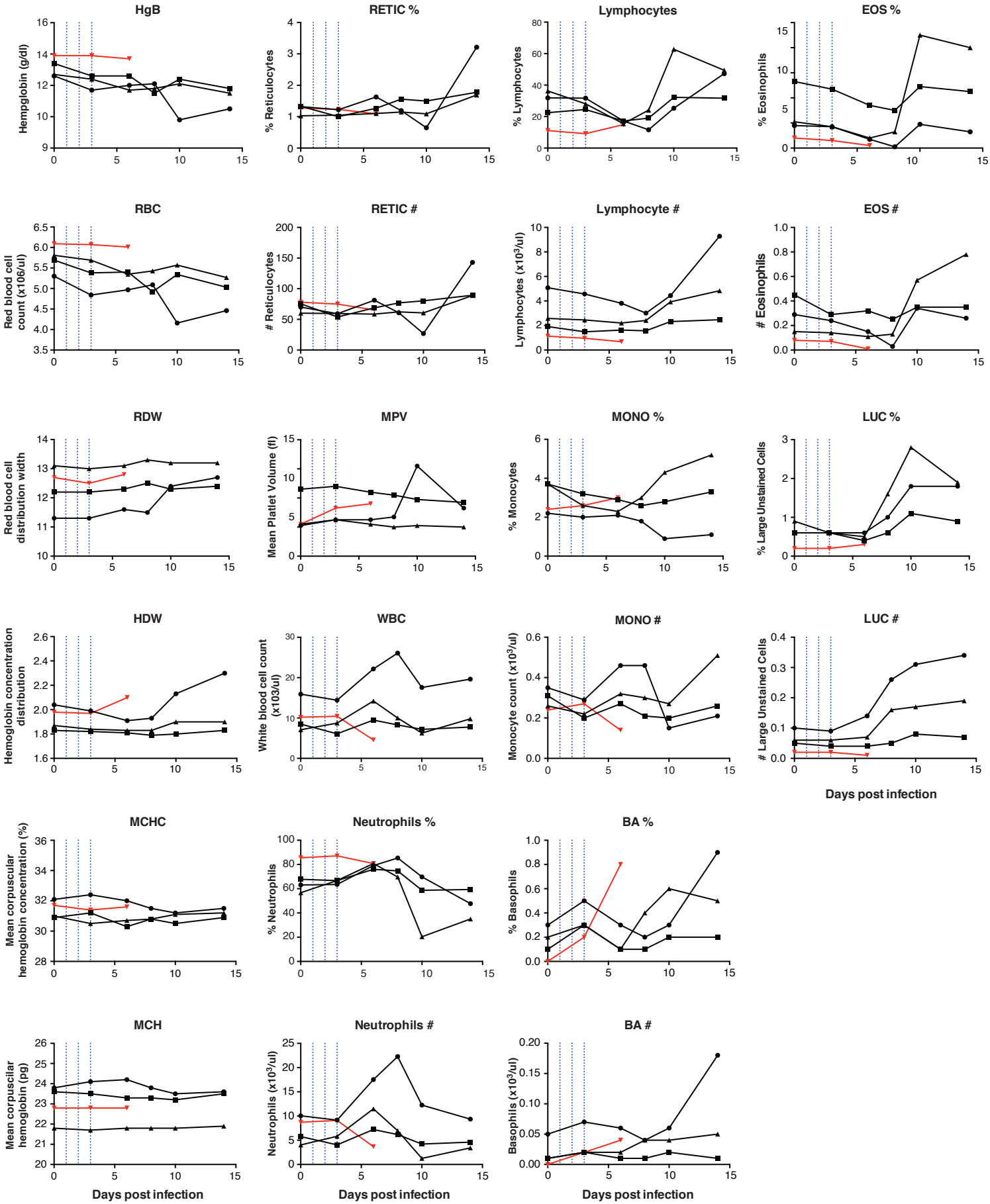
Figure S9



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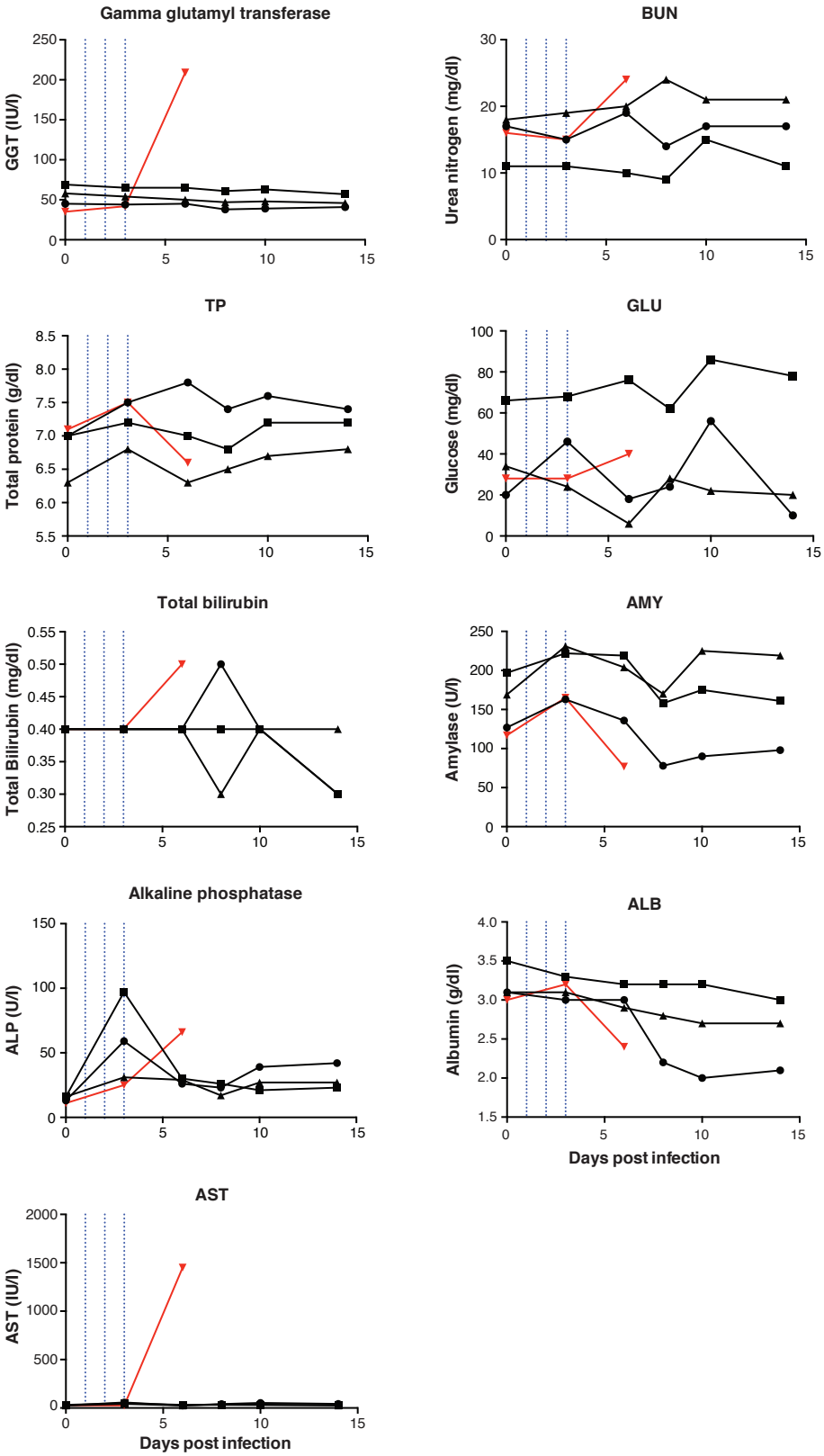
273 **Figure S9**. **Additional hematology data from passive transfer of mAb114.**

274

275

Figure S10


Gamma glutamyl transferase
GGT (IU/l)
BUN
Urea nitrogen (mg/dl)
TP
Total protein (g/dl)
GLU
Glucose (mg/dl)
Total bilirubin
Total Bilirubin (mg/dl)
AMY
Amylase (U/l)
Alkaline phosphatase
ALP (U/l)
ALB
Albumin (g/dl)
AST
AST (IU/l)
Days post infection
Days post infection

276 **Figure S10. Additional serum chemistries from passive transfer of mAb114.**

277

278

Figure S11

Cage 1

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	1	1	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NE	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	V	N	N	V	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 2

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	O	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 3

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Biscuit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fruit Consumption	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urine Output	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Condition of Stool	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cough	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Primary Euthanasia Criteria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cage 4

Day	0	1	2	3	4	5	6
Biscuit Consumption	0	1	0	0	0	0	2
Fruit Consumption	0	0	0	0	0	0	2
Urine Output	Y	Y	Y	Y	Y	Y	N
Condition of Stool	0	0	0	0	0	0	3
Cough	N	N	N	N	N	N	N
Facial Edema	N	N	N	N	N	N	N
Rash	0	0	0	0	0	0	0
Rash/ Hemorrhage at Venipunture Site	0	0	0	0	0	0	0
Bleeding	N	N	N	N	N	N	N
Motor Funtion	0	0	0	0	0	0	0

Observations

Biscuit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Fruit Consumption	0=>=50%, 1=26-49%, 2=0-25%, NE=Not Evaluated
Urine Output	Y=Yes, N=No, NE=Not Evaluated
Condition of Stool	0=normal, 1=not fully formed, 2=liquid, 3=none, NE=Not Evaluated
Cough	Y=Yes, N=No
Facial Edema	Y=Yes, N=No
Rash	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Rash/ Hemorrhage at Venipunture Site	0=none/barely visible, 1=mild rash <=10% body surface, 2=moderate rash 11-50% body surface, 3=severe rash >50% body surface, NE=Not Evaluated
Bleeding	N=None, F=Facial, V=Vaginal, O=Other, NE=Not Evaluated
Motor Funtion	0=normal, 1=mild coordination, dysfunction trouble manipulating items, 2=moderate dysfunction trouble grasping, moving, 3=severe dysfunction (uncoordinated actions), NE=Not Evaluated

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Figure S11. Clinical observation scoring from passive transfer of mAb114.

Data from multiple observations each day post-exposure are presented as the maximal level observed for that day.

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